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**INCORPORATION OF PYRIMIDINES
AND FLUOROPYRIMIDINES
INTO RNA AND DNA**

An experimental study in rats with liver tumor



Christian Erichson



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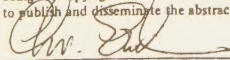
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Title and subtitle Incorporation of pyrimidine and fluoropyrimidines into RNA and DNA. An experimental study in rats with liver tumor.			
Abstract In an experimental model with a N-methyl-N-nitrosoguanidine induced adenocarcinoma of the colon transplanted into the liver of Wistar rats, the incorporation of pyrimidines and fluoropyrimidines into target molecules of tumor and normal tissues was studied. Different administration routes were compared. Hepatic artery infusion of ^3H-uridine showed higher labelling of tumor RNA than portal, intravenous or intraperitoneal infusion. The ratio of ^3H-uridine and ^3H-FURd labelling of tumor RNA to that of normal tissues was also greater by the arterial route. The fluoropyrimidines were incorporated into tumor RNA in higher amounts than the natural counterparts. Fluorinated pyrimidine nucleosides (FURd, FdURd) were incorporated into tumor RNA to a higher degree than the corresponding base (FURA). Different FURA dosages and infusion times showed increased incorporation of FURA into tumor RNA with increasing dosages. However, the ratio of FURA incorporation into RNA of tumor to that of normal tissues was more favourable with an intermediate dosage. A UTP depressing effect of N-phosphonacetyl-L-aspartate (PALA) and D-glucosamine was recognized in tumor and liver but no increase in tumor RNA labelling was achieved by combining ^3H-FURd with these antiprimidines. The labelling of liver RNA was on the other hand increased five-fold compared to ^3H-FURd alone indicating an increased liver toxicity. Hepatic artery ligation (HAL) and intraportal infusion of ^3H-orotic acid showed decreased labelling of tumor RNA after three and five days compared to controls. This does not support the hypothesis of portal revascularization of the tumor after HAL. Hepatic artery infusion of degradable starch microspheres (DSM) concomitant with ^3H-uridine or ^3H-uracil showed increased tumor RNA labelling compared to administration of either metabolite alone. Thus DSM promote the anabolism of a RNA precursor in tumor when given together through the hepatic artery.			
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Christian Erichsen, M.D., Department of Surgery, Lund University, S-221 85 Lund, Sweden.

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INCORPORATION OF PYRIMIDINES AND
FLUOROPYRIMIDINES INTO RNA AND DNA

An experimental study in rats with liver tumor

Christian Erichsen

leg läkare

Lund

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From the Department of Surgery, University of Lund,

S-221 85 LUND, Sweden

INCORPORATION OF PYRIMIDINES AND
FLUOROPYRIMIDINES INTO RNA AND DNA

An experimental study in rats with liver tumor

by

Christian Erichsen

Lund 1986

Dedicated to

my wife Ulla and my son Morten,

my parents Inger and Finn

For their love and support.

The thesis is based on the following papers:

- I EFFECTS OF ADMINISTRATION ROUTES ON THE UPTAKE OF URIDINE AND 5-FLUOROURIDINE INTO AN ADENOCARCINOMA TRANSPLANTED TO RAT LIVER. Erichsen C, Christensson P-I, Eriksson G, Yngner T, Jönsson P-E and Stenram U. J Surg Oncol, 1986 (In press).
- II INCORPORATION OF PYRIMIDINES AND 5-FLUOROPYRIMIDINES INTO NORMAL TISSUES AND AN ADENOCARCINOMA TRANSPLANTED INTO THE LIVER IN RAT. Christensson P-I, Erichsen C, Jönsson P-E and Stenram U. J Cancer Res Clin Oncol, 1985;110:95-97.
- III EFFECT OF DOSAGE AND INFUSION TIME OF 5-FLUOROURACIL ON DNA AND RNA OF NORMAL TISSUES AND AN ADENOCARCINOMA TRANSPLANTED TO RAT LIVER. Erichsen C, Christensson P-I, Jakobsson B, Jönsson P-E and Stenram U. Submitted for publication.
- IV EFFECT OF PALA AND D-GLUCOSAMINE ON URACIL NUCLEOTIDES AND THE INCORPORATION OF 5-FLUOROURIDINE INTO NORMAL TISSUES AND AN ADENOCARCINOMA TRANSPLANTED INTO THE LIVER IN RAT. Erichsen C, Christensson P-I, Jakobsson B, Eriksson G, Yngner T, Jönsson P-E and Stenram U. Submitted for publication.
- V EFFECT OF HEPATIC ARTERY LIGATION ON THE INCORPORATION OF ^3H -OROTIC ACID INTO AN ADENOCARCINOMA TRANSPLANTED TO RAT LIVER. Erichsen C, Christensson P-I, Jönsson P-E and Stenram U. Europ J Surg Oncol, 1986 (In press).
- VI HEPATIC ARTERY ADMINISTRATION OF DEGRADABLE STARCH MICROSPHERES; II. EFFECTS ON INCORPORATION OF URIDINE AND URACIL INTO RNA OF AN ADENOCARCINOMA TRANSPLANTED TO RAT LIVER. Teder H, Erichsen C, Christensson P-I, Jönsson P-E, Lewan L and Stenram U. Res Exp Med, 1986 (In press).

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ABBREVIATIONS

AMP, ADP, ATP	Adenosine mono-, di-, triphosphate
ASF	Acid soluble fraction
ASP	Aspartate
BW	Body weight
CA	Carbamoyl aspartate
CMP, CDP, CTP	Cytidine mono-, di-, triphosphate
CP	Carbamoyl phosphate
Cy	Cytosine
d...	deoxy.. (ex. dUrd = deoxyuridine)
D-Glc	D-glucosamine
DNA	Deoxyribonucleic acid
dpm	Degradation per minute
DSM	Degradable starch microspheres
F...	Fluoro... (ex. FUra = fluorouracil)
Fol	Folate
Gln	Glutamine
Glu	Glutaminic acid
HAL	Hepatic artery ligation
mCi	Millicurie
NG-W	Nitroso-guanidine-Wistar
PALA	N-phosphonacetyl-L-aspartate
PCA	Perchloric acid
PP	Pyrophosphate

PRPP	Phosphoribosylpyrophosphate
Q	Quinone
RNA	Ribonucleic acid
SE	Standard error of the mean
TCA	Trichloroacetic acid
Thy	Thymine
TMP, TDP, TTP	Thymidine mono-, di-, triphosphate
UPA	Ureidopropionic acid
Ura	Uracil
Urd	Uridine
UMP, UDP, UTP	Uridine mono-, di-, triphosphate
μ kat	microkatal

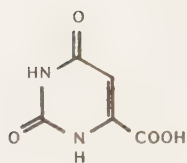
INTRODUCTION

Historical background

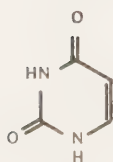
Fluoropyrimidines were synthesized and introduced by Duschinsky and Heidelberger 1957 (Duschinsky et al 1957). The rationale for the development of these drugs was that uracil was incorporated into the nucleic acids of a liver tumor to a considerably greater extent than into the normal liver (Rutman et al 1954, Heidelberger et al 1957). It was expected that the fluorosubstitution of the five carbone-bound hydrogen of uracil (Fig I) would yield the cytotoxic effect which later has been confirmed in several studies (Heidelberger 1981). The mechanisms through which the fluoropyrimidines exert their cytotoxicity are, however, still controversial although their interaction with nucleic acids is most important.

Pyrimidines and antipyrimidines

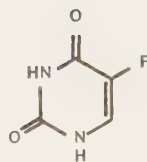
The nucleic acids are composed of nucleotides derived from pyrimidines and purine bases, sugar molecules and phosphoric acids. Changes in nucleic acid synthesis and composition are expected to have deleterious effects on cell function and survival. In mammalian cells *de novo* pyrimidine synthesis involves six cytoplasmic enzymes (Fig II. Jones 1980). Inhibitors of these enzymes are of great interest as they might act synergistically with fluoropyrimidines in clinical cancer treatment. The first and rate limiting enzyme of *de novo* pyrimidine synthesis is carbamoylphosphate synthetase. This enzyme shows high activity in proliferating cells of normal and tumor tissues (Tatibana & Shigesada 1972). Acivicin, a chlorinated amino acid, is an antagonist to this enzyme but *in vivo* the inhibition is rapidly reversed (Kensler et al 1982). In various tumor lines a



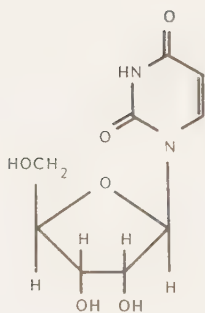
Orotic acid



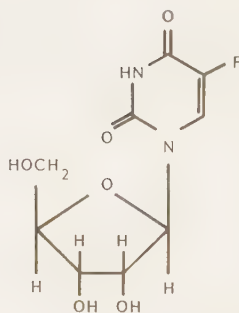
Uracil



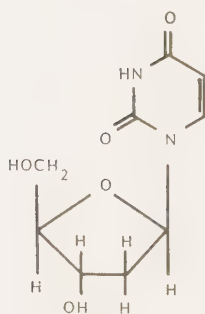
5-Fluorouracil



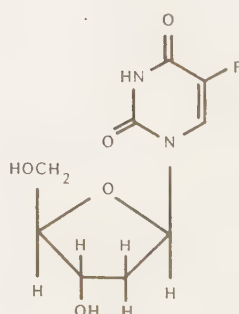
Uridine



5-Fluorouridine



Deoxyuridine



5-Fluorodeoxyuridine

Fig. 1

feed back inhibition of carbamoylphosphate synthetase by UTP has been found (Ito et al 1970). This is also reflected in intact liver by an immediate increase of UMP formation de novo after depletion of the UTP pool in the cytoplasm by UTP consuming sugar analogues (Keppler et al 1970). This explains why lethal UTP depletion can not be achieved by the use of a sugar analogue as the sole antipyrimidine. The carbamoylphosphate synthetase has a high affinity for the phosphate donor ATP (Mori & Tatibana 1975). This makes the enzyme sensitive to hypoxic conditions. The second enzyme of de novo pyrimidine synthesis is aspartate transcarbamylase. It catalyzes the reaction which yields carbamoyl-aspartate. A powerful inhibitor of this enzyme is PALA (Collins & Stark 1971). In vitro this antipyrimidine produces an almost complete inhibition of pyrimidine synthesis. However, cells resistant to PALA have been recognized and these cells appear to have a greatly increased aspartate transcarbamylase or carbamoyl-phosphate synthetase activity (Swryd et al 1974, Kensler et al 1982). Dihydro-orotate is the third enzyme which together with the two former enzymes are located on the cytoplasmic multienzyme pyr 1-3. Fluoroorotate is known to be an inhibitor of this enzyme. Dihydroorotate oxidase is the next enzyme and is located in the mitochondrion. It transfers electrons and is intimately connected to acceptors of the respiratory chain and therefore sensitive to hypoxia (Miller et al 1968, Löffler 1980). The two last enzymes are located on the second multienzyme pyr 5-6. These are orotate phosphoribosyltransferase and orotidine monophosphate decarboxylase (reviewed by Jones 1980). High activity of these enzymes are found in proliferating tissues (Pausch et al 1972). Inhibitors of the latter enzyme are pyrazofurin, ribonucleosidephosphates of allopurinol and aza-uridine (reviewed by Keppler & Holstege 1982).

De novo pyrimidine synthesis

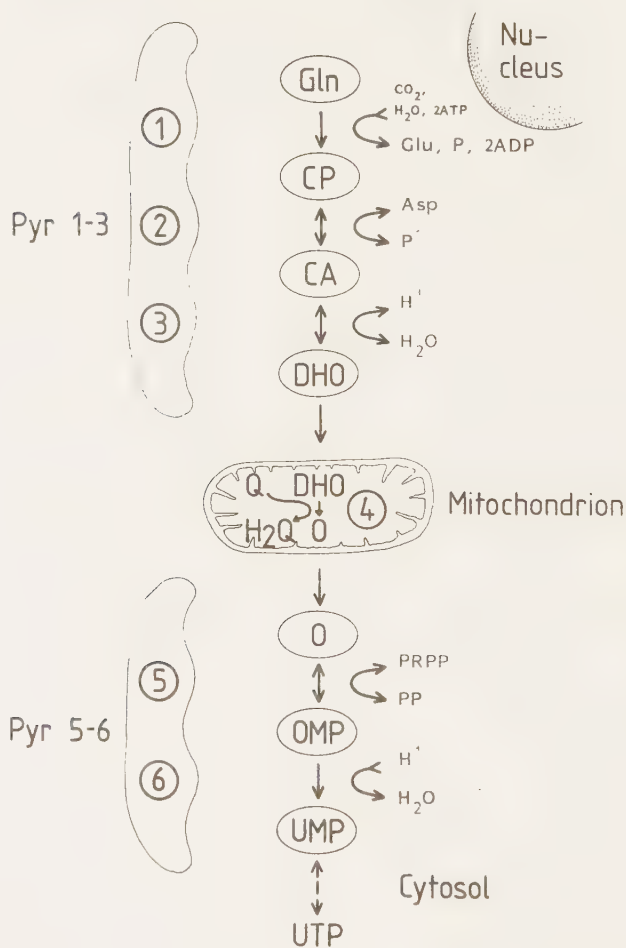


Fig. II 1. Carbamoylphosphate synthetase 4. Dihydroorotate dehydrogenase
 2. Aspartate transcarbamylase 5. Orotate phosphoribosyltransferase
 3. Dihydroorotase 6. Orotate monophosphate decarboxylase

Asp, CA, CP, DHO, O, OMP, PRPP, Pyr 1-3, Pyr 5-6, Q, UMP, UTP:
 See abbreviation.

Inhibitors of de novo pyrimidine synthesis have demonstrated effects on the growth of experimental tumors both in vitro and in vivo (reviewed by Skoda 1975, Kensler et al 1982, Rozenzweig et al 1979, Johnson et al 1976, 1978). Clinically a limited and temporary effect has been recognized in leukemia patients after aza-uridine treatment (reviewed by Skoda 1975) and in colon cancer patients treated with PALA as a single drug (Erlichmann et al 1979, Rubin et al 1981). For the treatment of an adenocarcinoma PALA seems to be a very interesting inhibitor for several reasons. The antitumor potential of the drug has been clearly demonstrated in murine colon carcinoma and Lewis lung carcinoma (Tsuboi et al 1977, Johnson et al 1978). Its activity early in de novo pyrimidine synthesis might be of importance for the availability of several enzymes. Increased availability of orotate phosphoribosyltransferase which acts on uracil and FUra producing UMP and FUMP might be an important factor in the synergism between fluoropyrimidines and PALA. Apparently PALA stimulates the utilization of the preformed pyrimidine pathway because in resistant cell lines of Lewis lung carcinoma the uridine kinase activity was significantly elevated compared to sensitive cell lines after exposure to PALA (Kensler et al 1981). This increase in uridine kinase activity may contribute to the synergistic effect of PALA and FUra on tumor cell growth (Ardalan et al 1980). However, it has been claimed that in spite of optimal inhibition of de novo pyrimidine pathway by PALA, the effect of the combination PALA/FUra would be similar to that of FUra alone if the tumor is sufficiently capable of salvaging pyrimidine nucleotides (Jayaram et al 1979). Both normal and tumor cells are able to utilize preformed pyrimidines and this has been named the salvage pathway of pyrimidines (Sneider & Potter 1969). Thus circulating pyrimidines in blood provide a source for nucleotide synthesis in various organs (Moyer et al 1981). It has been

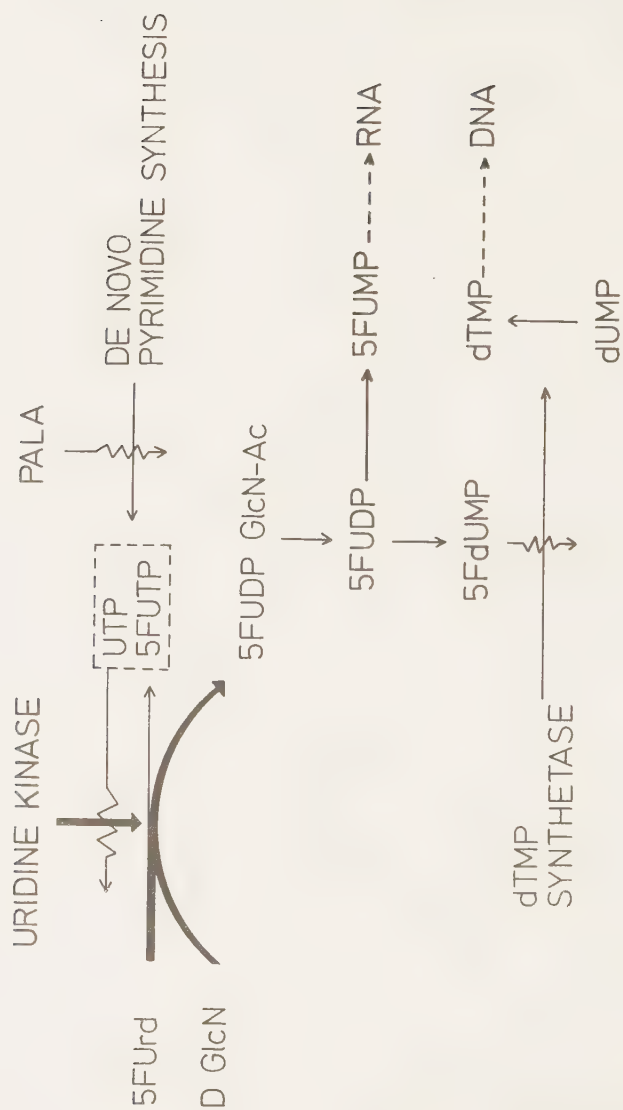


Fig. III

claimed that a depletion of the UTP pool of the cell results in reduction of RNA, DNA and protein synthesis and even cell death (Keppler 1978). UTP reduction also enhances the activity of uridine kinase, the rate limiting enzyme for the formation of FUMP from FUrđ. In hepatoma cells increased incorporation into RNA for FUrđ was recognized after depletion of the UTP pool (Holstege et al 1978). However, in vivo inhibition of de novo pyrimidine synthesis is insufficient to deplete the UTP pool in most tissues (Keppler et al 1977, Moyer & Handschumacher 1979). The salvage pathway of pyrimidine metabolism can be inhibited by D-galactosamine or D-glucosamine, thereby contributing to the decrease of the UTP pool (Keppler 1978, Holstege et al 1982). In rats bearing hepatoma, a combined treatment with D-galactosamine, PALA and FUrđ increased the incorporation of FUrđ into tumor RNA compared to the incorporation after FUrđ alone. A high percentage of ascites hepatoma bearing rats also became tumor free after combined treatment (Anukarahanonta et al 1980). Synergism by such combinations could also be attractive for the treatment of adenocarcinomas (Fig III). Because enzymes of the galactose pathway are abundant in hepatomas as well as in the liver, D-galactosamine has high affinity to these tissues. Depletion of uridine nucleotide pools by trapping in the form of UDP sugar derivatives has been proposed to explain the liver toxicity of D-galactosamine (Decker et al 1971, reviewed by Decker 1974). For the purpose of UTP trapping in an adenocarcinoma located in the liver, D-glucosamine appears more attractive. Even in high doses it does not cause liver toxicity (Keppler et al 1970). In cell suspensions of colon cancer and TA 3 mammary cancer, D-glucosamine reduced the UTP pool (Holstege et al 1982, Krug et al 1984). In a previous study with an adenocarcinoma in rat liver, pretreatment with PALA and D-glucosamine potentiated the inhibitory effect of FUrđ on tumor growth

Metabolism of Pyrimidines and Fluoropyrimidines

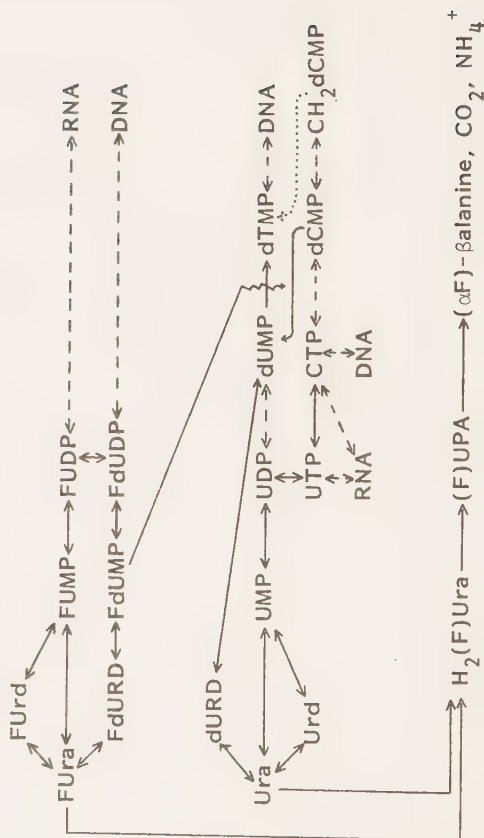


Fig. IV

(Erichsen et al 1982). The cytotoxic effects of D-glucosamine have been confirmed in malignant tumors both in vivo and in vitro (Quastle & Cantero 1953, Bekesi et al 1969, Friedman & Skehian 1980). However, for the fluoropyrimidine treatment of adenocarcinoma, the synergistic role of D-glucosamine and PALA has not yet been established. Therefore it should be important to evaluate the effect of PALA and D-glucosamine on the UTP pool and find out if there exists an increased incorporation of a fluoropyrimidine into the cells of an adenocarcinoma.

Fluoropyrimidines

Tumors are affected in different ways by fluoropyrimidines (Heidelberger et al 1958). FdUMP was found to be a powerful inhibitor of thymidylate synthetase (Cohen et al 1958) and some authors have proposed that to be the main mechanism whereby fluorinated pyrimidines inhibit tumor growth (Heidelberger 1981, Spears et al 1984). Thymidylate synthetase catalyzes the methylation of the natural substrate dUMP to dTMP for the synthesis of DNA and, as a consequence, inhibition of this enzyme will affect DNA synthesis (Klubes et al 1978). In this reaction a co-factor N^5-N^{10} -methylene-tetrahydrofolate is converted to dihydrofolate which is then regenerated by dihydrofolate reductase. The availability of this co-factor in different tissues might be important for the formation of a thymidylate synthetase - FdUMP - N^5-N^{10} -methylene-tetrahydrofolate complex and consequently also for DNA synthesis and the incorporation of fluoropyrimidines into DNA as well. In vitro experiments with responsive and non-responsive tumor lines have shown that maximum enzyme inhibition of thymidylate synthetase by FdUMP was achieved only in the presence of an exogenous co-factor (Houghton et al 1981). Fluoropyrimidines have been recog-

nized to be incorporated into DNA of normal and tumor cells in vitro (Danenberg et al 1981, Ingraham et al 1982). In vivo FUra was shown to be incorporated into murine bone marrow DNA and the incorporation level was closely associated with the inhibition of DNA synthesis and toxicity (Schuetz et al 1984, Sawyer et al 1984). On the other hand it was early recognized that fluoropyrimidines after conversion to FUTP become incorporated into RNA of normal and tumor cells (Chaudhuri et al 1958). This has later been reported for a variety of animal and human cell lines (Glazer & Hartman 1980, Glazer & Lloyd 1982).

Fluoropyrimidines are incorporated into all classes of RNA including ribosomal, transfer and messenger RNA but in tumor cells FUra is mainly found in nuclear RNA (Mandel 1981). A consequence of fluoropyrimidines being incorporated into RNA is an impaired maturation of ribosomal RNA (Stenram & Willén 1970, Wilkinson et al 1975) and a decreased transcription rate of RNA (Iapalucci-Espinoza & Franze-Fernandez 1982, Eriksson & Stenram 1984). The cytotoxicity of fluoropyrimidines has been correlated to the degree of incorporation into RNA in several tumor lines including colon carcinoma (Spiegelmann et al 1980, Glazer & Lloyd 1982). However, even the incorporation into ASF was recognized to be closely correlated to the cytotoxicity in vitro (Meisler & Squeglia 1985). Thus there are several mechanisms by which the fluoropyrimidines affect the tumor cells (Fig IV). More information can probably be gained if their incorporation into different target molecules of tumor and normal tissues is explored in a tumor model which integrates important elements of the clinical situation. Under physiological conditions pyrimidine bases appear to be degraded rather than converted to nucleotides in most mammalian tissues (Canellakis 1957, Lea et al 1974). Deleterious effects on the cell are thus mostly prevented by catabolism

(Canellakis 1957, Cooper et al 1972). It has been suggested that tumor tissues have reduced capacity to degrade pyrimidines (reviewed by Wasternack 1980). The transport system used by uracil and FUra allows rapid equilibration across the cell membrane. However orotate permeation across the cell membrane has been suggested to proceed via diffusion (Wohlhueter et al 1980). Evidence for an active transport of orotate has also been reported and orotate does not seem to be degraded to any significant extent (Yngner 1979). The intra- and extracellular nucleoside concentrations equilibrate within a minute and the subsequent formation of pyrimidine nucleoside phosphates is catalyzed by specific kinases. They are thereby trapped within the cell since the plasma membrane is impermeable for nucleotides. The cleavage of pyrimidine nucleosides to bases results in transport into the extracellular space or increased catabolic degradation in the cell (reviewed by Keppler & Holstege 1982). Thus it is important to evaluate differences in metabolism of pyrimidine and fluoropyrimidine bases and nucleosides in order to choose appropriate drugs in cancer therapy. Furthermore incorporation pattern of different pyrimidines and fluoropyrimidines must be screened before studies with pyrimidine potentiators and rescue agents can be performed in the present model.

Tumor vascularization and drug administration

The vascular supply of primary and secondary liver tumors has been extensively investigated both in experimental animals and humans (Breedis and Young 1954, Honjo and Matsumura 1965, Ackerman 1974, Sundqvist 1980, Carlsson 1982, Liu 1984). In experimental tumors the vascularization varies with histopathological type. Honjo and Matsumura demonstrated by injecting dyed gelatine solutions that cholangiocarcinomas were supplied entirely from the hepatic artery, while

hepatomas has both arterial and portal blood supply (Honjo and Matsumura 1965). Morphologic studies with microfil have revealed that most human secondary tumors in the liver have both arterial and portal blood vessels although the arterial vascularity prevails. These patterns were also recognized in an experimental adenocarcinoma using the same technique (Lin 1984). Arterio- and portography of an NG-W induced adenocarcinoma inoculated into the liver of rat showed an arterially hypovascular central zone and a hypervascular periphery in tumors with a diameter 3-8 mm. Tumors with diameters up to 12 mm had a more homogenous arterial vascularization and larger tumors revealed a vascular distribution similar to the small tumors. Portal vessels could be recognized in the tumor periphery but also some leading in to the central portions (Carlsson 1982, Lin 1984). However, the studies present a static picture of the tumor vascularity which do not tell us anything about the microcirculatory relationship between the arterial and portal system. A significantly higher arterial blood flow is found in the periphery compared to the central parts of the same tumor (Sundqvist 1980). This blood flow distribution is related to central necrosis of the tumor (Sundqvist 1980, Carlsson 1982). There might exist mechanisms which permit exchange of arterial and portal blood in the periphery of liver tumors. From measurements of pressure in the microvessels of the liver we know that the pressure of terminal portal and hepatic venules are about 5 and 1 cm H₂O respectively. Although the hepatic arteriolar pressure is in the range of 40-50 cm H₂O, both arterial and portal blood can pass into the sinusoids. Sinusoid sphincters might be an explanation (Bloch 1970, McCusley 1966, reviewed by Rappaport 1976). At the tumor border arterioles are known to be innervated by adrenergic nerves which can not be found in intratumoral vessels (Sundqvist 1980). Thus sphincter mechanisms might regulate the contribution of either the

arterial or the portal blood supply to tumors in the liver. Therefore conclusions can not be drawn from morphologic studies for identification of the most suitable administration route for cytotoxic drugs.

An important aim of all chemotherapeutic effort must be to deliver as much as possible of the drug to the tumor and as little as possible to normal tissues. Pharmacokinetic studies of fluoropyrimidines have been performed using different administration routes (Collins et al 1980, Enslinger et al 1978, Frei III et al 1981). The extraction of FdUrd and FUra of liver after hepatic artery infusion to patients has been estimated as being about 90 % and 19-51 % respectively. After hepatic artery infusion of FUra the systemic arterial concentration was about 25 % and that of the venous was about 60 % of the corresponding concentrations after peripheral venous infusion (Enslinger et al 1978). Because of the predominantly arterial supply of liver tumors, it has been assumed that secondary liver tumors are exposed to the same drug concentration that exists in the hepatic artery (Frei III et al 1981). From studies on pharmacokinetic models it has been claimed that the regional exposure of an agent with linear kinetics depends on the elimination of the agent in the rest of the body and the blood perfusion through the target region (Chen and Gross 1980). Fluoropyrimidines have a short half-life and a high total body clearance as well as an extensive hepatic clearance which should be favourable factors for regional administration (reviewed by Enslinger & Gyves 1985). However, their pharmacokinetics are non-linear and therefore factors concerning uptake and metabolism of fluoropyrimidines can not be exactly predicted in linear pharmacokinetic models (Collins et al 1980, Goldtman et al 1981, Cano et al 1981). Therefore it will be more interesting to study the incorporation of these drugs into target molecules than studying their concentrations in different parts of the circulation.

Intraperitoneal infusion of fluoropyrimidines has been proposed to be an important route of administration of fluoropyrimidines. Compared to arterial and intravenous infusion high intraportal and intraperitoneal drug concentrations have been found after intraperitoneal administration (Speyer 1985). This could be an alternative route if local recurrence and peritoneal dissemination or hepatic metastases exists. Mixing of arterial with portal blood supply has been the proposed reason for good drug exposure to small intrahepatic metastases (Collins 1984). Concomitantly a low systemic drug exposure should be expected as the intrahepatic drug metabolism would remove most of the fluoropyrimidines in the first passage. However, at the same time an increased liver toxicity has to be considered. This could not be confirmed in a prospective randomized trial of intravenous versus intraperitoneal FUra administration to patients with advanced primary colorectal cancer (Sugarbaker et al 1985). A reduced hepatic toxicity was seen in patients who received FUra intraperitoneally. There was no difference in survival, however, and only the incidence of peritoneal carcinomatosis was reduced significantly. For estimation of the efficacy of the intraperitoneal administration route for treatment of liver metastases with fluoropyrimidines an experimental model designed for this purpose could give valuable information.

Systemic infusion of fluoropyrimidines has been commonly used but with limited clinical response (reviewed by Lockich 1985). This might be explained by an insufficient drug exposure to the tumor cells because of high extraction by the liver and extrahepatic organs. After continuous intravenous infusion the total body clearance of FUra is in the range of 2-6 l/min. The clearance is limited by the organ blood flow and in the liver this is 1.5 l/min. Thus probably more than

50 % of the FURa metabolism occurs outside the liver with intravenous infusion (Collins et al 1980, reviewed El Sayed & Sadée 1983). Consequently normal cells should be unnecessarily exposed to fluoropyrimidine cytotoxicity after intravenous infusion. Bone marrow suppression was found to be more pronounced after rapid intravenous infusion whereas gastrointestinal toxicity was dose limiting by continuous infusion (Moertel & Shutt 1972, Fraile et al 1980). Thus a high plasma concentration of FURa in the systemic circulation seems to increase bone marrow toxicity but different tissues might anabolize the fluoropyrimidine in different ways. A murine study recently demonstrated enhanced antitumor effect in AKR leukemia but reduced in L-1210 leukemia when FURa was given as a 24 hour infusion compared to when the drug was given as intravenous bolus injection (Santelli and Valeriote 1986). Further information concerning scheduled sensitivity might be gained from experimental studies performed in vivo particularly with regard to the incorporation of fluoropyrimidines into different tissues.

Pharmacokinetic studies of FURa infused into the hepatic artery have proposed this administration route to be more favourable compared to the intravenous (Ensminger et al 1978, 1983). It has been demonstrated that the total body clearance of FURa decreased with increasing doses given via continuous infusion. However, the hepatic extraction was also reduced with increasing doses which means loss of regional selectivity (Ensminger et al 1978). Doses and schedules which combine a high drug exposure to the liver tumor with a maximum tolerated dose to extrahepatic tissues should be selected if there is also extrahepatic tumor. In a prospective randomized study of hepatic artery infusion versus intravenous FURa administration no significant differences in response

rates of survival was recognized (Grafe et al 1979). However, only one course of intraarterial FUra treatment was performed which then was continued with the intravenous route. Further investigation of the potential benefit of intraarterial administration can be done experimentally by the use of different dosages given with different lengths of infusion. An optimal schedule for intraarterial fluoropyrimidine infusion would be most important for clinical application. Nevertheless a large proportion of patients with liver metastasis will respond to intraarterial chemotherapy with fluoropyrimidines (Patt et al 1980, Balch et al 1983, Ekberg et al 1986).

As indicated by Ensminger a reduced blood flow rate through the hepatic artery should theoretically increase the concentration of an agent in the arterial vascular bed (Ensminger 1985). Degradable starch microspheres seem to be a useful tool to reduce arterial blood flow. At high doses they are capable of totally blocking the hepatic arterial blood flow but in about 75 % of patients it decreases by 80 % and arterio-venous shunting occurs (Aronsen et al 1979, Dakhil et al 1992). The DSM used for intraarterial administration (Spherex^R) have a mean diameter of 40-45 μm and are degraded by alpha amylase. Their ability to slow or arrest the blood flow is dependent on both the diameter and the number of microspheres infused (reviewed by Lindberg et al 1984). Different factors might theoretically influence the synergistic effect of the microspheres on the regional uptake and anabolism of fluoropyrimidines. First extrahepatic shunting occurs which might reduce the regional selectivity (Ziessman et al 1983). This might induce unpleasant side effects from sensitive organs i.e. lungs and bone marrow but no significant side effects were observed in a clinical trial combining DSM and FUra (Aronsen et al 1979). Secondly the ischemic effects of slowing the

blood flow should have an important impact on several metabolic steps leading to the incorporation of fluoropyrimidines into the target molecules. Repeated administration of DSM via the hepatic artery in rats demonstrated only a slight increase in serum liver enzymes and no effects to liver cells. However, the liver has a dual blood supply and increased portal blood flow was measured with xenon technique in the rat after repeated embolization (Lindell 1977). DSM infused into the hepatic artery of rats with a liver tumor showed a more prominent reduction in the tumor blood flow compared to that of normal liver (Lindell 1977). Consequently the influence on the tumor energy charge should be anticipated to be more prominent and thus more deleterious to the tumor than the liver. A negative influence on the incorporation of fluoropyrimidines into the cell targets could be expected. Co-administration of ^{14}C -FUra and DSM into the liver artery in rats demonstrated increased total ^{14}C -activity in the liver compared to that of FUra infusion alone (Teder et al 1978). This reflects the retention of the drug or its degradation products in the liver either localized in the vascular bed or in the liver cells but no conclusions can be drawn regarding the drug anabolism. Recently different cytostatics including FUra have been administered through the hepatic artery to patients with liver metastases and a similar increase in tumor tissue drug concentration was observed (Löffler et al 1985). However, the same criticism as before must be mentioned. The amount of drug incorporation into anabolic products i.e. RNA, DNA and nucleotides must be considered a more reliable criterion in the evaluation of studies dealing with combined modalities of ischemia and drug administration.

Ischemia is known to produce certain changes in the cell which have a deleterious effect on cell metabolism. After one hour of complete liver ischemia

in rats the ATP content of the liver was reduced to less than 1/10 of the normal value and there was a severe deprivation of protein synthesis. After one hour of hepatic artery ligation only the ATP content was significantly reduced but less than after complete ischemia (Fornander et al 1985). Experimental liver tumors exposed to hepatic dearterialization revealed impaired oxidative phosphorylation with mitochondrial changes and low activity and nucleotide specificity of membrane bound ATP-ase enzymes (Sugahara et al 1975). These changes indicate a considerable decrease in tumor metabolism after hepatic artery ligation. Markowitz 1952 was the first to suggest the use of HAL in the treatment of patients with liver metastases. Since then this has been used by several others (Nilsson 1966, Almersjö et al 1966, Petrelli et al 1984). Microscopic examinations of tumors after HAL revealed central necrosis but vital tumor cells were observed in the periphery (Almersjö et al 1966, Nilsson & Zettergren 1967). A suggestion is that these cells survive because of nutritional support from the portal vein (Taylor 1981). Studies of the flow dynamics with the xenon technique showed a 35 % decrease in the normal liver perfusion after HAL whereas liver metastases lost 95 % of their blood supply (Taylor 1979). However, xenon injection into the portal vein of patients before HAL indicated little if any perfusion into metastatic tissue whereas good perfusion was demonstrated after HAL (Taylor 1981). One clinical study has evaluated HAL and intraportal FUra infusion. Improved survival and better tumor response compared to a non-treated control group was reported. No benefit was found with HAL and arterial FUra perfusion and with portal vein infusion alone (Taylor 1978). However, this trial included only 24 patients allocated into four groups in which the HAL + portal infusion-group had the least extensive disease. No definite conclusions can therefore be drawn from this study but further research should be designed to

evaluate the importance of portal infusion of fluoropyrimidines in combination with arterial occlusion. Development of collaterals to the tumor has been recognized about five days after HAL and intermittent occlusion of the hepatic artery has been proposed in order to avoid this development (Bengmark & Rosengren 1970, Persson et al 1984).

Experiments with nuclei and nucleoli from ischemic liver cells have demonstrated that after restoration of the blood supply the RNA synthesis of recovering cells will increase above normal levels for at least 24 hours (Schiaffonati 1978). Similar conditions of reversible ischemia and RNA anabolism might be expected in the periphery of tumors exposed to HAL. The fluoropyrimidines are known to exert most of their effect by incorporation into RNA and thus increased susceptibility to fluoropyrimidines should be expected in this part of the tumor. After HAL the portal blood flow to the tumor was thought to increase (Almersjö et al 1968) and vasoactive substances promoting this effect might be released by the ischemia (Taylor 1981). After HAL the intratumoral pressure might be reduced. Such changes should be in favour of an improved portal blood supply and possibly a portal vascularization of the tumor. However, ischemia produces swelling and enlargement of cells which might compress the smooth veins in the tumor periphery. Furthermore microscopic examination of metastatic tumor not exposed to ischemia has demonstrated an avascular zone in the tumor periphery which corresponds to plates of liver cells with compressed sinusoids (Lin et al 1984). It is reasonable to anticipate that edema in this region negatively influence the portal blood supply of the tumor. Thus important relations exist between ischemic cell reactions, blood flow and drug metabolism which influence the efficiency of fluoropyrimidine administration.

PURPOSE AND AIMS OF THE STUDY

Among all the different cytostatic agents fluoropyrimidines have been the most extensively used in treatment of liver metastases from gastrointestinal cancers. The clinical role of fluoropyrimidines alone or in combination with other agents has been established. However, it is of utmost importance to further investigate these drugs in experimental models which mimic the clinical situation in order to establish alternative treatment schedules for future use in humans. This study was planned

- to further explore the effects of different administration routes on the pyrimidine and fluoropyrimidine incorporation into target molecules of tumor and normal tissues.
- to compare the incorporation pattern of different pyrimidines and fluoropyrimidines in tumor and normal tissues after administration through the hepatic artery.
- to investigate the effect of dosage and infusion time of FUra on DNA and RNA and thereby identify an optimal dose schedule with respect to therapeutic index.
- to evaluate the effect of PALA and D-glucosamine on the UTP pool and determine if this promotes an increased incorporation of FUrd into tumor and normal tissue.

- to study the effect of hepatic artery ligation on the incorporation of a pyrimidine precursor (^3H -orotic acid) into tumor tissue after intraportal administration.
- to explore the extent of portal revascularization of tumor after hepatic artery ligation.
- to determine whether pyrimidine anabolism in tumor increase after hepatic artery ligation.
- to investigate the effect of arterially administered degradable starch microspheres on the incorporation of a pyrimidine base and a pyrimidine nucleoside into target molecules of tumor and normal tissues.

METHODOLOGICAL CONSIDERATIONS

Several *in vitro* experiments using different cell lines including adenocarcinoma cells of the human colon have demonstrated that pyrimidines and fluoropyrimidines interfere with RNA and DNA and cause cell death (Glazer & Lloyd 1982, Schuetz et al 1984, Parker & Klubes 1985). Most experiments with fluoropyrimidines *in vivo* have been focused on the metabolism, concentration and distribution of the substances in different normal and tumor tissues (Klubes et al 1978, Ardalan et al 1978, Houghton et al 1979, 1980), their effects on tumor growth (Heidelberger et al 1958, Anukarahanonta et al 1980, Erichsen et al 1982) and influence on ribosomes and cell morphology (Stenram & Willén 1970). Other important factors to consider are tumor site and vascularization as well as the route of drug administration. Therefore it is relevant to use an experimental model which mimics the clinical situation. In such a model more parameters can be standardized and controlled and essential information can be gained with regard to the relationship between pyrimidines and fluoropyrimidines and their target molecules of tumor and normal tissues. The variations in enzyme activities and metabolic pathway between different species must be considered, however, and results obtained experimentally have to be confirmed clinically. Fundamental results from animals are necessary when clinical drug schedules are designed because drug toxicity is very similar in animals and man (Freireich et al 1966).

Differences in enzyme activities have been demonstrated between rats and man. Thymidine phosphorylase responsible for splitting of dThd and Urd shows high activity in the human spleen but lacks activity in the rat spleen (Liermann et al

1984). Theoretically this might imply differences in the capacity of rats and humans to catabolize infused pyrimidines and fluoropyrimidines. However, significantly higher thymidine phosphorylase activity has been found in the plasma of cancer patients compared to control persons though in both groups the amount of uridine catabolized was negligible (Pauly et al 1977). Even in the same type of tumor, variation in enzyme activity has been recognized, as reported for the uridine kinase activity of human colon cancer (Sköld 1963, Ahmed et al 1981). The measured serum level of amylase in the rat is approximately 100 and that of humans only $3 \mu\text{kat/l}$ (Lindberg et al 1984). This should imply a protracted degradation of DSM in man compared to the rat and an enhanced ischemic effect might be expected in man. A negative effect on drug uptake after combined infusion might result but, on the other hand, a protracted degradation might also promote better drug retention in the organ and thus enhanced uptake into the tumor.

The tumor used in our model showed a homogenous growth. The growth pattern and vascularization of the tumor is well known (Carlsson 1982). Rats in which extrahepatic tumor spread was seen were not used. Tumor cell soiling was minimized by careful technique with change of instruments between each animal and the application of a piece of Spongostan (gelatine) on the liver surface when the needle was withdrawn. The energy charge of liver is suppressed by anesthetics. In this study we used the halothane - $\text{N}_2\text{O}-\text{O}_2$ gaseous mixture as this affects the energy charge less than other anesthetics (Christensson & Eriksson 1985). The short time interval between cutting off a piece of tissue until it was frozen in liquid nitrogen as well as the storage of specimens at -80°C prevented degradation of nucleotides and macromolecules by enzyme activity (Munro and Fleck 1966).

To investigate the effects of different administration routes on the incorporation of pyrimidine and fluoropyrimidine into target molecules (paper I), ^3H -uridine and ^3H -FUrd were used. These metabolites are known to be rapidly incorporated into the RNA of mammalian tissues (reviewed by Keppler & Holstege 1982) and FUrd is an interesting drug to explore in combination with antipyrimidines (Anukarahanonta et al 1980). Even ^3H -orotic acid and its fluoroanalogue could be used. However, their transport mechanism through the cell membrane is controversial (Wohlhueter et al 1980, Yngner 1979) and the latter has also the disadvantage of being very expensive. In paper I and II tracer doses were used to demonstrate drug distribution in order to prevent the interference with pyrimidine pools which might occur by using therapeutic doses. For evaluation of different dosages and infusion schedules (paper III) the isotope was mixed with cold FUra. Three dosages were evaluated. A therapeutic dose related to the body weight, an intermediate dose related to the weight of the liver and a small dose were studied. In the first liver passage high extraction of pyrimidines seems to occur by arterial administration. However, a dose liver weight relationship should be expected for optimal drug saturation. On the other hand the extra-hepatic extraction might be important even at lower dosage if the infusion rate is rapid (Ensminger et al 1978). In paper IV PALA was given in a dosage known to act synergistically with FUrd in hepatomas (Anukarahanonta et al 1980). D-glucosamine was used in a dosage which previously has shown growth inhibitory effects on tumor cell suspension in vitro (Friedman & Skehan 1980) and even reduced the UTP pool of sarcoma 180 and ascites hepatoma cells (Bekesi & Winzler 1969, Keppler et al 1970).

For the evaluation of portal infusion and HAL (paper V) ^3H -orotic acid was used as model substance. The efficiency of this pyrimidine precursor was clearly demonstrated in paper III. This is also in accordance with previous studies with this isotope (Eriksson 1983). Three time intervals between HAL and the evaluation of nucleotide, RNA and DNA content and their radioactive labelling of intraportally infused ^3H -orotic acid were studied the first five days. During this time the tumor might be expected to be nourished predominantly by the portal vein. Between five and ten days after HAL the arterial collaterals should probably have developed (Bengmark & Rosengren 1970, Carlsson et al 1981, Stridbeck et al 1984) and the portal contribution to tumor blood supply might again be of minor importance. Thus evaluation of the target molecules was also performed ten days after HAL.

Degradable starch microspheres might be given in several dosages (reviewed by Ensminger & Gyves 1985). However, 12 mg DSM administrated into the hepatic artery in rats concomitant with a sodium pertechnetate as a drug model showed a favourable retention of the pertechnetate in the liver compared to lower dosages of DSM (Teder et al 1985). In paper VI uracil and uridine were used as model substances in order to evaluate if differences exist between a base and a nucleoside regarding incorporation into macromolecules during co-administration with microspheres. The amount of incorporation of the drugs into the target substances was evaluated after 3 and 60 minutes. These time intervals were chosen because a pyrimidine precursor can be transformed into phosphorylated ribosides within 3 minutes (Eriksson 1983) and 90 % of a tracer dose of a pyrimidine nucleoside, incorporated into the acid-insoluble fraction, can be found in the RNA after 60 minutes (Hinrichs et al 1964). However, the

microspheres might influence cell metabolism and the optimal time interval for evaluation of maximum drug incorporation into the target molecules can not be predicted. The chosen interval should, however, be sufficient for the evaluation of the microsphere effect on drug uptake.

For estimation of ASF and RNA in the different tissues the method of Munro and Fleck was employed and, for DNA, the method of Scott as modified by Hinrichs (Munro & Fleck 1966, Scott et al 1956, Hinrichs et al 1964). Most methods used for nucleic acid determination are based on either strong ultraviolet absorption of the bases, specific colour reactions for the pentoses or on determination of phosphorus. However, the ultraviolet absorption is primarily used because this is not dependent on specific standards as is the colour procedure which also interferes with many carbon hydrates. Since only 80 % or less of the phosphorus in the RNA fraction can be accounted for by ribonucleotides, this method is not generally used (reviewed by Munro and Fleck 1966). Recently a method using chaotropic reagent (guanidine-thio-cyanate and LiBr) was described. With this method nucleases are rapidly inactivated and RNA selectively precipitated. Also high molecular weight DNA could be recovered from the LiBr-soluble supernatant by selective precipitation (Krawetz et al 1986). However, the methods of Munro and Fleck as well as Scott as modified by Hinrichs are well established in our laboratory. Consequently they were found suitable for the determination of the nucleic acids in the present work.

According to these methods the nucleic acid analysis was carried out in three steps. First the ASF containing small molecules i.e. free nucleotides, carbohydrates, inorganic phosphate and organic phosphorous compounds of low

molecular weight were removed. This is an important step because for instance failure to remove adenine nucleotides is known to result in approximately 10 % overestimation of the RNA content in the rat liver (Munro and Fleck 1966). For precipitation of ASF, RNA and DNA cold acid (PCA) was used. A high concentration of PCA must be avoided because the recovery of RNA is affected by increasing concentrations. An advantage of PCA to TCA is that there is no absorption in ultraviolet light by PCA and the following nucleic acid determination which involves ultraviolet absorption will not be influenced (Munro and Fleck 1966). The second step is the separation of the nucleic acids. Alkali is used to extract RNA from the pellet and thereby separate it from DNA, which is resistant to alkali. The alkali concentration and time of extraction chosen in this work have been suggested by Munro and Fleck who regard this procedure to be sufficient for extracting all the RNA from mammalian tissues in an acid soluble form. A higher alkali concentration or longer time of extraction increase the risk of making tissue proteins acid soluble resulting in interference with RNA by ultraviolet absorption (the third step). To rule out protein contamination the measurements are performed at different wavelengths. The wavelengths used correspond to the maximum and minimum ultraviolet absorption of RNA and proteins (Munro and Fleck 1966). The radioactivity of RNA and the ASF were correlated to the DNA content rather than to the weight of the tissues because the degree of tissue hydration will affect organ weight but not the cellular DNA content.

For separation of ribonucleotides isotachopheresis is a simple and rapid method. Other methods which might be employed are high pressure liquid chromatography (HPLC), paper electrophoresis and column chromatography. These methods have

various disadvantages of column packing and long regeneration time, tedious procedure and requirement of concentrated solutions or large volumes (Eriksson 1983). Thus isotachophoresis appears to be the most attractive method for the following experiments (paper IV).

MATERIAL AND METHOD

Animals

Inbred female Wistar rats weighing 145-250 g were used in all experiments. The rats were delivered from Anticimex, Stockholm, Sweden (paper I-V) and from Møllegaard-Hansen, Avslaboratorium, AS Denmark (paper VI). The rats were kept individually and fed on standard food pellets and tap water ad libitum.

Tumor

The experimental tumor used was an N-methyl-N-nitrosourea-induced adenocarcinoma of rat colon (NG-W), obtained from the Wallenberg Research Laboratory in Lund (Steele and Sjögren 1974). The tumor was propagated by intraperitoneal transplantation in inbred Wistar rats. For this purpose frozen tumor was thawed in a water bath over 15 minutes at room temperature. Then it was minced mechanically and a trypsin buffer solution was added. One to two milliliters of this cell suspension were then injected into the peritoneal cavity. Every time a new frozen tumor was used it was given a T-number. Tumors with T-number 20-22 were used in paper I-VI. After two or three weeks of intraperitoneal growth, the tumor was ready for use in experiments and standardized cell suspensions could be prepared. Tumor was given a generation number after each intraperitoneal passage. Tumors with generation numbers 24-35 were used in papers I-VI.

For preparation of a tumor cell suspension a macroscopically homogenous piece of tumor was taken out under ether anesthesia and the tumor was cleaned from supportive tissues, necrotic and hemorrhagic parts. Under sterile conditions a

tumor cell suspension was prepared using trypsin. Vital counts were made in a hemocytometer by adding trypan blue. The concentration of the tumor cell suspension was 1.0×10^6 cells/0.1 ml. Inoculation of the liver was performed with the rat under ether anesthesia and through a midline incision. Using a syringe with a thin needle 0.1 ml of the solution (1.0×10^6 cells) was injected under the capsule on the ventral surface into the periphery of the central liver lobe (Carlsson 1982, Carlsson et al 1983). To prevent leakage of tumor cells after withdrawal of the injection, a piece of Spongostan was pressed against the liver surface until hemostasis was obtained. Twelve rats were inoculated at a time. Measurements of tumor size were performed with a Vernier calipers and the tumor volume was calculated according to the formula $a \times b^2/2$ where a is the largest and b the smallest diameter (Carlsson et al 1983). Ten days after inoculation the tumor volume was approximately 0.25 cm^3 .

Anesthesia

The inoculation was performed under ether anesthesia in all experiments. At laparotomy and when the rats were sacrificed anesthesia was given with 4 % halothane in a gaseous mixture of 2 l N_2O and 1 l O_2 /min, in a plastic jar. Thereafter the anesthesia was continued by a mask and the concentration of halothane was reduced to 1 %.

Catheter placement

At laparotomy eight to ten days after inoculation, catheters (Intramedic PE 10 or PE 50, Clay Adams, Parsippany, N.J.) were placed into the gastroduodenal artery, the portal vein, a femoral vein or into the peritoneal cavity (I-IV, VI). The tip of the artery catheter was stretched to fit into the lumen of the vessel. It

was anchored to the vessel by a 7-0 silk ligature (Leivestad & Malt 1973). The hepatic artery was ligated with 7-0 silk ligature (V). The catheters were tunneled subcutaneously and brought out in the distal part of the tail. Thereafter the rats were placed in individual cages and the catheters were connected to an infusion pump (except VI). Injectomat R 50, Fresenius, Bad Homburg, FRG, which infused 5 ml 0.9 % NaCl solution/24 hours (I) and type 1850 Braun AG, FRG, giving 1 ml of the same solution/24 hours (II-V) and a STC 521 pump (Terumo, Japan) for different infusion rates were used. Heparin 10 I.U./ml was added to prevent catheter occlusion or thrombosis.

Isotopes and drugs

The following isotopes were used; 5,6-³H-uracil (20-42 Ci/mmol), 5,6-³H-FUra (16.5 Ci/mmol), 5,6-³H-uridine (38.7-38.9 Ci/mmol), 5,6-³H-FUrd (16.5-17.8 Ci/mmol), 6-³H-deoxyuridine (18 Ci/mmol), 5,6-³H-FdUrd (14.7 Ci/mmol) and 5-³H-uronic acid (20 Ci/mmol). They were purchased from New England Nuclear Corporation (Boston, Mass.).

Unlabelled uracil and uridine were delivered from Sigma Chem. Co. St Louis MO. Uracil 6.45 mg/ml and uridine 6.45 mg/ml dissolved in 0.05 M Tris-HCl buffer solution, pH 8.8, were mixed separately with ³H-uracil (1.25 mCi/kg BW) and ³H-uridine (1.25 mCi/kg BW) respectively before use. All chemicals were analysed.

The ³H-fluoropyrimidines were delivered in 70 % ethanol and were freeze-dried and dissolved in sterile distilled water before use. A recovery of about 90-95 % of the radioactivity was found. The isotopes were immediately used in order to

minimize selfdecomposition (Sheppard et al 1974). D-glucosamine and N-phosphonacetyl-L-aspartate (PALA) were delivered from Calbiochem (La Jolla, Cal.).

Degradable starch microspheres (Spherox^R) were obtained from Pharmacia AB, Uppsala, Sweden. They are cross-linked and made by emulsion polymerization of a potato-starch hydrolysate. The microspheres swell in water and exhibit gel characteristics. They are delivered in an aqueous solution and have a mean diameter of 40 μm (range 20-60 μm). The DSM are degraded by alpha-amylase. The degradation time is related to the degree of crosslinking and the diameter. In vitro at an amylase concentration of 4 $\mu\text{kat/l}$ half the microsphere mass is dissolved in 12 minutes; at 25 $\mu\text{kat/l}$ the time needed is 6 minutes (paper VI).

Tissue sampling

In the tracer experiments the rats were sacrificed 90 minutes after the tracer was infused. In experiments with different doses and lengths of infusion time they were sacrificed 1.5, 3 or 24 hours after start of the FUra infusion. In the DSM-studies they were sacrificed three or 60 minutes after the drugs were administered. The tissue sampling for UTP analysis was performed one hour and 24 hours respectively, after the end of the infusions with D-glucosamine and PALA was over. In consecutive order liver, tumor, right kidney, spleen, a piece of small intestine, femoral bone marrow and thymus were removed and immediately frozen in liquid nitrogen. The liver was thawed to about 0° C and pressed through a plastic tube with 1 mm bottom holes to remove connective tissue (Willén 1970) and thereafter mixed by stirring, refrozen and stored at -80° C for later analysis. For isotachopheretic analysis a part of the liver was rapidly

freeze-clamped and frozen in liquid nitrogen. Tumor cleaned from liver parenchyma was cut into small pieces with a pair of scissors, then mixed by stirring and frozen in liquid nitrogen. In paper IV some tumors were freeze clamped in liquid nitrogen for isotachophoretic analysis. Spleen, kidney, thymus, small intestine and bone marrow were quickly dissected out and cleaned from connective tissue and then frozen in liquid nitrogen. The distal 10 cm of the ileum was dissected free from the mesentery, resected and rinsed with distilled water before freezing. Both femurs were cut out and opened at both ends. The bone marrow was pressed out with a straightened metal paper clip and then placed on a small piece of paper and put into an aluminium foil, which was frozen in liquid nitrogen. All samples were stored at -80°C .

Extraction and analysis

The acid soluble fraction (ASF) was extracted according to Munro and Fleck. 200 mg liver, tumor and splenic tissues were homogenized in a Colworth "stomacher" (England) with 12 ml of cold distilled water (4°C) for 5 minutes in a refrigerator bench. A volume of 2 x 5 ml was taken out and filled into centrifuge tubes which contained 2.5 ml of 0.6 M cold perchloric acid (PCA). The contents were mixed and kept in an ice bath for 10 minutes. They were centrifuged in a SS 34 rotor (Sorvall, USA) and placed in a RC2B or RC5 centrifuge (both from Sorvall, USA) at $4 \times 340\text{ g}$ for 10 minutes. The residues were washed with 5 ml of 0.2 M cold PCA and centrifuged as above, twice. Then they were used for analysis of RNA and DNA as described below. Before the estimation of ASF, the supernatant was diluted to 50 ml with water. Kidney and small intestine tissues (200 to 300 mg) were placed in a precooled aluminium chamber containing liquid nitrogen. Then the tissue was hammered into powder and weighed in a crystal balance (CER) on

a Fine Balance Sauter 420/R3 (Sauter, GFR). It was homogenized in 6 ml of distilled water with a knife homogenizer (MSE, England) for 2.5 minutes. The homogenate was then diluted with another 6 ml of distilled water and two samples of 5 ml were treated as described above for liver, tumor and spleen. Thymus was frozen, hammered, weighed and homogenized just as kidney and small intestine but the homogenate was not diluted. A volume of 2 x 2.5 ml of homogenate was taken and filled into a centrifuge tube containing 1.25 ml of 0.6 M PCA and then treated as above. The specimen of bone marrow was taken out of the aluminium foil and excess of paper was cut away. Paper and the tissues were weighed and placed in a centrifuge tube with 1.5 ml of distilled water. The paper was removed, dried and weighed. The sample was mixed with 0.75 ml of 0.6 M PCA and treated as described for liver, tumor and spleen with the exception that only 50 % of the remaining volumes needed for the extraction were used. The RNA was extracted according to Schmidt and Thannhauser as modified by Munro and Fleck (Munro and Fleck 1966). After extraction of the ASF, the residue was further extracted for RNA using 4 ml of 0.3 M KOH, however, for the extraction of the bone marrow RNA only 2 ml of the solution was used. The extraction was performed in a shaking bath at 37° C for one hour. Then the samples were put into an ice bath and 2.5 ml (for bone marrow only 1.25 ml) of 1.2 M PCA was added. Thereafter the samples were mixed, centrifuged and washed in the manner described for the ASF. The DNA was extracted by the technique of Scott (Scott et al 1956) as modified by Hinrichs (Hinrichs et al 1964). After the RNA extraction the residue was further extracted with 5 ml of 1 M PCA at 60° C for 15 minutes. Thereafter it was placed in an ice bath for 10 minutes and centrifuged at 7 700 g. This procedure was repeated once. For estimation of ASF, RNA and DNA a Gilford 230

spectrophotometer (USA) was used. The extinction of the supernatants was measured at 232, 260 and 280 nm. According to Munro and Fleck an extinction of 1 000 at 260 nm corresponds to 32 µg RNA/ml extract (Munro and Fleck 1966). This value is also valid for UV-absorbing substances in the ASF (Christensson personal communication). ASF and RNA values in the tables of paper I-VI refer to the amount of UV-absorbing substance in these fractions. For control of purity the side wavelengths are used (Stenram & Wilander 1973). For measurement of the radioactivity in the ASF, RNA and DNA, 1 ml of the extracts and 10 ml Instagel (Packard, USA) were used. The analysis were performed with a Packard (Downers Grove ILL) Tri Carb CD 300 liquid scintillation system. In paper II the dpm of the target nucleotides and RNA was divided by 2.22 x the specific activity to give the amount of administered substance from the labelled batch. In paper III this amount had to be multiplied with the amount of cold FUra to give the amount of given substance incorporated into the fractions. The extraction for isotachophoretic analysis was performed with cold 0.7 M PCA and 30 % methanol. It was then neutralized with 2 M KOH (Eriksson 1980). The samples were frozen and stored at -80°C for later analysis. A LKB 2127 Tachophor (Sweden) with a column coupling system according to Eriksson was used for the analysis (Eriksson 1983).

Statistical analysis

Student's two-tailed t-test was employed.

Administration routes (paper I)

Ten days after the tumor cell inoculation, a catheter (PE10) was placed into the gastroduodenal artery, the portal vein, a femoral vein or into the peritoneal

cavity. Altogether thirteen rats (3-4 in each group) were used to investigate the effect of the different administration routes on the incorporation of ^3H -uridine into the acid soluble fraction and RNA of tumor and normal tissues. ^3H -uridine (1.25 mCi/kg BW) was infused by the catheters 36 hours after they were inserted. Ninety minutes later the animals were anesthetized and the tissue sampling was performed. The same procedure was used when five rats were given ^3H -FUrD (1.25 mCi/kg BW) by the gastroduodenal artery.

Pyrimidines and Fluoropyrimidines (paper II)

Fourty-two rats were used for the investigation of the incorporation of different pyrimidines and flurorpyrimidines into tumor and normal tissues. A catheter (PE10) was placed into the gastroduodenal artery. The following day, tracer doses of ^3H -labelled pyrimidines and fluoropyrimidines were infused through the catheter (1.25 mCi/kg BW). The rats were anesthetized in a halothane- N_2O - O_2 atmosphere when the tissue sampling was performed as earlier described.

Different 5-FUra dosages and infusion times (paper III)

For the investigation of the effect of FUra dosage and infusion time on DNA and RNA of tumor and normal tissues, 84 rats had the catheter placed into the gastroduodenal artery. The catheter was connected to an infusion pump (1 ml 0.9 % NaCl/24 hours). Three days later the rats were randomized to three different dose schedules of FUra, 300, 1 200 and 24 000 nmol respectively and administered through the catheter with different lengths of infusion time (0.5, 2 and 24 hours). The rats were sacrificed 24 hours after start of the infusion, except rats subjected to short infusions which were sacrificed 1.5 or 3 hours after the infusions (see the tables). The tissue sampling was then performed.

PALA and D-glucosamine (paper IV)

The effect of PALA and D-glucosamine on the UTP pool and on the incorporation of FUr^d into tumor and normal tissues was investigated. Twenty-three rats were used. The catheter (PE10) was placed in the gastroduodenal artery. First PALA (200 μ mol/kg BW), and D-glucosamine (10 mmol/kg BW) were given separately to each three rats and both drugs together to four rats. The rats were sacrificed one hour after administration of D-glucosamine and 24 hours after PALA. A part of the liver and the whole tumor were sampled for nucleotide analysis. In a second experiment five rats were pretreated with PALA and D-glucosamine in the same doses as above. Twenty-three hours after the administration of PALA they were given D-glucosamine and after one more hour a tracer dose of ³H-FUr^d (0.15 mCi) was infused. Four controls received 0.9 % NaCl alone. After a labelling period of 90 minutes the tissue sampling was performed.

Hepatic artery ligation and intraportal infusion(paper V)

The effect of hepatic artery ligation on the incorporation of ³H-orotic acid into tumor and normal tissues when administered by the portal vein, was studied in 42 rats. In 11 ligated rats the catheter was placed in a femoral vein or into the peritoneal cavity. In the hepatic artery ligated rats 0.25 mCi ³H-orotic acid was given through the catheter after one, three, five or ten days. Eleven ligated rats were given the same dose tracer by the femoral vein or by the intraperitoneal route. The tissue sampling was performed after a labelling period of 90 minutes as described above.

Degradable starch microspheres (paper VI)

Fifty-one rats with the catheter placed into the gastroduodenal artery were used. In a first experiment urecil (15 mg/kg BW) together with ³H-leucine was

mCi/kg BW) were injected alone or mixed with 12 mg of the DSM. In a second experiment comparable dosages of uridine together with ^3H -uridine were injected alone or mixed with the DSM. 0.8-0.9 ml of the substances were infused through the catheter over one minute. A loop around the common hepatic artery prevented retrograde flow. After a labelling period of 3 or 60 minutes the animals were sacrificed and the tissue sampling performed.

RESULTS

The influence of different administration routes on the incorporation of ^3H -uridine into the tumor ASF and RNA (paper I) is shown in table I. A more than two-fold higher incorporation into the tumor ASF was demonstrated by the intra-arterial administration compared to the others. The difference in specific radioactivity of RNA was even more pronounced with a ten-fold higher radioactivity of RNA by the hepatic artery compared to systemic administration and sixty-fold higher when compared to the intraperitoneal route. In the liver the labelling of the ASF was almost equal and high by all four administration routes (table I). The specific radioactivity of RNA in bone marrow and small intestine was low after infusion by the hepatic artery and the portal vein and high by the femoral vein. The ratio between the labelling of RNA of tumor to that of other tissues was greatest with the arterial route. ^3H -FUrd gave results very similar to those obtained with ^3H -Urd (table II).

In paper II tracer doses of FUra and FUrd gave greater labelling of the ASF of all tissues, especially liver and kidney, than the corresponding natural compounds. FdUrd gave greater labelling of this fraction in liver and kidney than deoxyuridine. FUra gave a substantially heavier labelling of tumor RNA than uracil. FdUrd gave a lower labelling than deoxyuridine of DNA, but a heavier labelling of RNA in all tissues except liver (Table III, III a). Orotic acid gave a heavier RNA labelling than all the other compounds in tumor, liver and kidney (table III).

In paper III there is from a general point of view an increasing incorporation of FUra into the ASF, RNA and DNA fraction with increasing doses with some

TABLE I

Effect of administration route on the incorporation of ^3H -uridine into ASF and RNA of tumor and normal tissues. s = specific activity.

The table gives the means $\pm$ SE.

	Route of administration	Tumor	Liver	Bone marrow	Kidney	Small intestine
sASF	Gastroduodenal art	5048 $\pm$ 1700	1808 $\pm$ 560	808 $\pm$ 186	1084 $\pm$ 343	602 $\pm$ 158
	Portal vein	1624 $\pm$ 330	1723 $\pm$ 77	1060 $\pm$ 85	1279 $\pm$ 61	798 $\pm$ 22
	Intraperitoneal	1449 $\pm$ 89	1191 $\pm$ 88	907 $\pm$ 45	1035 $\pm$ 79	1266 $\pm$ 195
	Femoral vein	1541 $\pm$ 17	1369 $\pm$ 87	1168 $\pm$ 101	1679 $\pm$ 133	1146 $\pm$ 133
sRNA	Gastroduodenal art	548 $\pm$ 211	65 $\pm$ 21	12.7 $\pm$ 5.6	19.3 $\pm$ 9.2	15.4 $\pm$ 5.0
	Portal vein	9.8 $\pm$ 2.2 ^a	79 $\pm$ 8.2	11.0 $\pm$ 1.0	14.7 $\pm$ 0.9	14.1 $\pm$ 0.8
	Intraperitoneal vein	7.4 $\pm$ 0.8 ^a	24 $\pm$ 5.1	43 $\pm$ 34	30 $\pm$ 13.7	74 $\pm$ 25
	Femoral vein	43 $\pm$ 0.2 ^b	30 $\pm$ 0.3	101 $\pm$ 15.9 ^b	142 $\pm$ 23	63 $\pm$ 18.1
$\mu\text{gASF}/\mu\text{gDNA}$	All routes	0.40 $\pm$ 0.03	1.33 $\pm$ 0.04	0.20 $\pm$ 0.001	0.72 $\pm$ 0.001	0.39 $\pm$ 0.01
$\mu\text{gRNA}/\mu\text{gDNA}$	All routes	1.35 $\pm$ 0.08	3.96 $\pm$ 0.09	0.62 $\pm$ 0.02	1.02 $\pm$ 0.02	0.77 $\pm$ 0.02

a : $p < 0.001$

b : $p < 0.01$

TABLE II

Ratio between dpm in RNA/ μ g DNA in tumor to each of the other tissues.

The table gives the means $\pm$ SE. n = number of rats.

	$\frac{^3\text{H-uridine}}{^3\text{H-FUrd}}$		$\frac{^3\text{H-FUrd}}$		
	Gastroduodenal artery	Portal vein	Intraperitoneal	Femoral vein	Gastroduodenal artery
n	3	3	4	3	5
<u>Tumor Liver</u>	1.6 $\pm$ 0.4	0.1 $\pm$ 0.02 ^b	0.1 $\pm$ 0.01 ^a	0.5 $\pm$ 0.03 ^c	3.2 $\pm$ 0.7
<u>Tumor Bone marrow</u>	66.1 $\pm$ 24.1	2.5 $\pm$ 1.3 ^b	1.7 $\pm$ 0.2 ^a	1.1 $\pm$ 0.2 ^a	89.7 $\pm$ 14.9
<u>Tumor Small intestine</u>	38.3 $\pm$ 14.6	1.4 $\pm$ 0.5 ^b	0.3 $\pm$ 0.1 ^a	1.5 $\pm$ 0.4 ^b	87.7 $\pm$ 20.7

a = p < 0.001 compared to the group given $\frac{^3\text{H-uridine}}$ by the arterial route.

b = 0.001 < p < 0.01

c = 0.01 < p < 0.02

TABLE III

Amount of given substance as femtomole in the acid-soluble fraction, RNA and DNA per mg DNA, corrected for nmole given dose, 90 min after administration of the precursor. The table gives the means $\pm$ SEM. n = number of rats.

Precursor	Variable	Tumor	Liver	Bone marrow	Small intestine	Kidney	n
Uracil	ASF	325 $\pm$ 42	686 $\pm$ 35	65.2 $\pm$ 3.7	143 $\pm$ 5	308 $\pm$ 8	6
	RNA	7.68 $\pm$ 1.07	23.2 $\pm$ 2.8	1.73 $\pm$.10	3.47 $\pm$.24	3.20 $\pm$.31	
	DNA	.866 $\pm$.157	4.29 $\pm$.74	1.16 $\pm$.07	1.09 $\pm$.06	.769 $\pm$.80	
FUra	ASF	953 $\pm$ 123 ^a	10301 $\pm$ 799 ^a	154 $\pm$ 6 ^a	374 $\pm$ 48 ^a	6605 $\pm$ 541 ^a	6
	RNA	225 $\pm$ 50 ^a	49.8 $\pm$ 6.4 ^a	2.98 $\pm$.27 ^a	14.1 $\pm$ 10.5	9.25 $\pm$ 1.70 ^b	
	DNA	.553 $\pm$.182	5.98 $\pm$ 1.68	.045 $\pm$.024 ^b	.771 $\pm$.435	.324 $\pm$.140	
Uridine	ASF	569 $\pm$ 64	1413 $\pm$ 187	68.5 $\pm$ 4.5	151 $\pm$ 6	382 $\pm$ 42	6
	RNA	160 $\pm$ 21	224 $\pm$ 49	2.8 $\pm$.10	4.99 $\pm$.31	7.62 $\pm$.96	
	DNA	9.72 $\pm$ 1.89	10.2 $\pm$ 1.5	2.21 $\pm$.23	1.44 $\pm$.07	1.18 $\pm$.09	
FUrd	ASF	2031 $\pm$ 288 ^a	12114 $\pm$ 908 ^a	139 $\pm$ 7 ^a	352 $\pm$ 29 ^a	6213 $\pm$ 560 ^a	5
	RNA	718 $\pm$ 84 ^a	262 $\pm$ 42	8.58 $\pm$ 1.41 ^a	11.2 $\pm$ 4.0	38.9 $\pm$ 4.8 ^a	
	DNA	2.83 $\pm$.40 ^b	8.80 $\pm$ 1.60	1.32 $\pm$.42	1.14 $\pm$.61	.811 $\pm$.280	
Deoxyuridine	ASF	1447 $\pm$ 70	4356 $\pm$ 103	360 $\pm$ 10	790 $\pm$ 41	1784 $\pm$ 27	6
	RNA	54.3 $\pm$ 10.4	133 $\pm$ 5	10.0 $\pm$ 1.1	11.0 $\pm$.6	8.37 $\pm$.47	
	DNA	1047 $\pm$ 222	47.3 $\pm$ 15.1	329 $\pm$ 27	52.9 $\pm$ 12.5	5.66 $\pm$ 1.29	
FdUrd	ASF	1667 $\pm$ 532	19360 $\pm$ 1402 ^a	202 $\pm$ 9 ^a	461 $\pm$ 16 ^a	10483 $\pm$ 648 ^a	7
	RNA	636 $\pm$ 85 ^a	265 $\pm$ 67	485 $\pm$ 70 ^a	140 $\pm$ 7 ^a	65.9 $\pm$ 9.1 ^a	
	DNA	20.4 $\pm$ 2.6 ^a	8.82 $\pm$ 1.96 ^a	23.2 $\pm$ 2.6 ^a	6.19 $\pm$.23 ^a	1.92 $\pm$.36 ^b	
Orotic acid	ASF	4991 $\pm$ 1212	41989 $\pm$ 4191	76.7 $\pm$ 12.9	171 $\pm$ 7	11955 $\pm$ 2345	6
	RNA	1163 $\pm$ 304	7379 $\pm$ 842	15.8 $\pm$ 4.7	16.1 $\pm$ 2.5	2060 $\pm$ 433	
	DNA	10.4 $\pm$ 2.5	173 $\pm$ 26	4.27 $\pm$.86	.153 $\pm$.054	43.9 $\pm$ 10.2	

a = p < 0.001 compared to corresponding natural substance.

b = 0.001 < p < 0.01

c = 0.01 < p < 0.02

TABLE III a

Ratio between DPM in RNA/mg DNA in tumor to liver, bone marrow and small intestine.

The same animals as in Table III. The table gives the means $\pm$ SEM.

	Uracil	FUra	Uridine	FUrd	Deoxyuridine	FdUrd
<u>Tumor</u> Liver	$.38 \pm .09$	4.50 ± 1.19^c	$1.05 \pm .35$	$3.17 \pm .68^c$	$.40 \pm .06$	$3.38 \pm .91^a$
<u>Tumor</u> Bone marrow	$4.51 \pm .72$	76.6 ± 20.2^c	58.1 ± 8.5	89.7 ± 15.0	$4.82 \pm .87$	$1.49 \pm .31^b$
<u>Tumor</u> Small intestine	$2.26 \pm .36$	608 ± 510	32.3 ± 4.5	88.9 ± 21.0^c	27.6 ± 5.7	$4.56 \pm .55^a$

a = p < 0.001 compared to corresponding natural substance.

b = 0.001 < p < 0.01

c = 0.01 < p < 0.02

differences among the tissues (table IV). For instance the ratio of incorporation into tumor RNA to normal tissue RNA is always higher, and thus more favourable, with a 1 200 nmol dose (table V) than with 24 000 nmol.

In paper IV the isotachophoretic analysis showed a value of 72 nmol/mg DNA of UTP in the liver of control rats. This value decreased by 35 % with PALA treatment and by 45 % with combined treatment with PALA and D-glucosamine. UDP-N-acetylhexosamine was 600 nmol/mg DNA in the control rats and increased 50 % following combined treatment with PALA and D-glucosamine and almost three-fold with D-glucosamine only ($0.001 < p < 0.01$). Low values were obtained in tumor tissue. However, UDP-N-acetylhexosamine increased from 115 nmol/mg DNA in control rats to 290 after D-glucosamine treatment. The effect of PALA and D-glucosamine on the incorporation of ^3H -FURd into the ASF, RNA and DNA fraction of tumor and other tissues after intraarterial administration is shown in table VI. There was no increase in the labelling of the ASF, RNA or DNA in the tumor, bone marrow or small intestine. In liver, PALA + D-glucosamine increased the labelling of RNA five-fold. In the kidney the labelling of the ASF was significantly reduced and that of RNA significantly increased after PALA and D-glucosamine pretreatment. The labelling of the DNA fraction was significantly increased in the liver.

TABLE IV

Amount of given FUra as picomole in the acid-soluble fraction, RNA and DNA/mg DNA. Labelling time is from start of infusion till sacrifice. The Table gives the mean $\pm$ standard error of the mean. The ratio of ultraviolet-absorbing substances (NTR) in the acid-soluble fraction and RNA to DNA is also given. n = number of rats.

Dose and infusion time	Labelling time	Variable	Tumor	Liver	Bone marrow	Small intestine	Kidney	n
300 nmole 30 min	90 min	ASF	436 $\pm$ 49	2736 $\pm$ 251	109 $\pm$ 17	282 $\pm$ 76	1262 $\pm$ 247	7
		RNA	55 $\pm$ 5	28 $\pm$ 7	7.7 $\pm$ 2.3	13.1 $\pm$ 5.3	7.8 $\pm$ 1.4	
		DNA	0.61 $\pm$ 0.08	3.3 $\pm$ 1.6	1.3 $\pm$ 0.1	1.5 $\pm$ 0.7	0.64 $\pm$ 0.14	
1200 nmole 30 min	90 min	ASF	1489 $\pm$ 370	10982 $\pm$ 3216	155 $\pm$ 30	362 $\pm$ 34	7727 $\pm$ 2566	7
		RNA	274 $\pm$ 58	46 $\pm$ 13	11.5 $\pm$ 7.3	5.1 $\pm$ 1.3	9.2 $\pm$ 1.3	
		DNA	2.2 $\pm$ 0.5	3.6 $\pm$ 0.7	3.7 $\pm$ 2.3	0.84 $\pm$ 0.14	0.53 $\pm$ 0.05	
24000 nmole 30 min	90 min	ASF	15637 $\pm$ 2851	223857 $\pm$ 13326	3022 $\pm$ 70	5995 $\pm$ 231	221907 $\pm$ 27497	4
		RNA	2677 $\pm$ 713	848 $\pm$ 125	205 $\pm$ 9	124 $\pm$ 12	298 $\pm$ 24	
		DNA	14.4 $\pm$ 3.7	84 $\pm$ 16	36 $\pm$ 1	8.4 $\pm$ 0.7	19.0 $\pm$ 2.8	
300 nmole 30 min	24 h	ASF	104 $\pm$ 5	303 $\pm$ 29	30 $\pm$ 2	49 $\pm$ 6	134 $\pm$ 11	6
		RNA	30 $\pm$ 13	9.2 $\pm$ 4.5	3.6 $\pm$ 1.9	1.2 $\pm$ 0.2	4.6 $\pm$ 2.5	
		DNA	1.0 $\pm$ 0.5	1.4 $\pm$ 0.9	2.0 $\pm$ 1.0	0.27 $\pm$ 0.04	0.45 $\pm$ 0.21	
1200 nmole 30 min	24 h	ASF	450 $\pm$ 34	1488 $\pm$ 70	138 $\pm$ 6	228 $\pm$ 11	594 $\pm$ 48	7
		RNA	86 $\pm$ 32	27 $\pm$ 5	7.5 $\pm$ 1.4	9.9 $\pm$ 3.8	5.9 $\pm$ 1.5	
		DNA	1.5 $\pm$ 0.6	4.7 $\pm$ 2.4	2.3 $\pm$ 1.0	0.78 $\pm$ 0.38	0.65 $\pm$ 0.35	
24000 nmole 30 min	24 h	ASF	11281 $\pm$ 953	29520 $\pm$ 1802	2935 $\pm$ 270	6164 $\pm$ 689	14277 $\pm$ 747	6
		RNA	2103 $\pm$ 836	979 $\pm$ 128	271 $\pm$ 29	221 $\pm$ 21	247 $\pm$ 15	
		DNA	24 $\pm$ 7	41 $\pm$ 5	37 $\pm$ 5	17.5 $\pm$ 1.6	10.7 $\pm$ 0.7	

TABLE IV continued

Dose and infusion time	Labelling time	Variable	Tumor	Liver	Bone marrow	Small intestine	Kidney	n
1200 nmole 2 h	3 h	ASF	1179 $\pm$ 118	7742 $\pm$ 1072	116 $\pm$ 10	449 $\pm$ 128	5400 $\pm$ 780	9
		RNA	333 $\pm$ 84	40 $\pm$ 7	5.3 $\pm$ 0.2	10.2 $\pm$ 3.4	9.0 $\pm$ 0.6	
		DNA	3.0 $\pm$ 0.6	6.7 $\pm$ 1.9	3.0 $\pm$ 0.2	1.8 $\pm$ 0.7	0.76 $\pm$ 0.04	
300 nmole 2 h	24 h	ASF	159 $\pm$ 16	388 $\pm$ 53	45 $\pm$ 7	71 $\pm$ 7	198 $\pm$ 32	7
		RNA	32 $\pm$ 9	18.0 $\pm$ 7.4	1.1 $\pm$ 0.2	2.5 $\pm$ 0.5	0.92 $\pm$ 0.43	
		DNA	1.3 $\pm$ 0.8	1.2 $\pm$ 0.7	0.34 $\pm$ 0.15	0.39 $\pm$ 0.09	0.21 $\pm$ 0.10	
1200 nmole 2 h	24 h	ASF	517 $\pm$ 55	1611 $\pm$ 142	163 $\pm$ 8.3	279 $\pm$ 30	718 $\pm$ 72	7
		RNA	251 $\pm$ 97	20 $\pm$ 4	6.4 $\pm$ 1.9	40.4 $\pm$ 4	4.6 $\pm$ 1.0	
		DNA	1.9 $\pm$ 0.6	0.81 $\pm$ 0.62	0.91 $\pm$ 0.33	0.39 $\pm$ 0.09	0.18 $\pm$ 0.11	
24000 nmole 2 h	24 h	ASF	8000 $\pm$ 980	23849 $\pm$ 2010	2231 $\pm$ 243	4294 $\pm$ 532	10204 $\pm$ 979	6
		RNA	1446 $\pm$ 517	953 $\pm$ 244	215 $\pm$ 39	181 $\pm$ 47	242 $\pm$ 52	
		DNA	78 $\pm$ 56	82 $\pm$ 38	133 $\pm$ 71	49 $\pm$ 25	27 $\pm$ 12	
300 nmole 24 h	24 h	ASF	136 $\pm$ 24	803 $\pm$ 166	37 $\pm$ 4	129 $\pm$ 31	362 $\pm$ 43	6
		RNA	10.8 $\pm$ 4.2	4.5 $\pm$ 2.5	3.6 $\pm$ 0.8	2.1 $\pm$ 1.2	1.1 $\pm$ 0.6	
		DNA	nm	nm	1.5 $\pm$ 0.3	nm	nm	
1200 nmole 24 h	24 h	ASF	522 $\pm$ 97	2980 $\pm$ 308	148 $\pm$ 23	286 $\pm$ 49	1647 $\pm$ 279	6
		RNA	91 $\pm$ 20	23 $\pm$ 2	5.5 $\pm$ 1.1	6.2 $\pm$ 1.6	6.0 $\pm$ 0.6	
		DNA	0.96 $\pm$ 0.19	1.9 $\pm$ 0.4	2.5 $\pm$ 0.4	0.80 $\pm$ 0.21	0.66 $\pm$ 0.24	
24000 nmole 24 h	24 h	ASF	7978 $\pm$ 1037	47448 $\pm$ 5437	2728 $\pm$ 263	5527 $\pm$ 579	26928 $\pm$ 4137	6
		RNA	689 $\pm$ 109	527 $\pm$ 86	159 $\pm$ 22	157 $\pm$ 42	198 $\pm$ 22	
		DNA	17.1 $\pm$ 2.6	60 $\pm$ 14	52 $\pm$ 6	19 $\pm$ 5	27 $\pm$ 27	
All groups		mgNT/mgDNA	0.30 $\pm$ 0.01	1.44 $\pm$ 0.02	0.19 $\pm$ 0.00	0.33 $\pm$ 0.01	0.68 $\pm$ 0.01	84
		mgRNA/mgDNA	1.00 $\pm$ 0.03	4.12 $\pm$ 0.05	0.58 $\pm$ 0.01	0.79 $\pm$ 0.01	0.99 $\pm$ 0.01	84

nm = not measurable

TABLE V

Tumor: normal tissue ratios with respect to labelling in the acid-soluble fraction and in RNA.

The Table gives the mean $\pm$ standard error of the mean. n = number of rats.

Dose and infusion time	Labelling time	Variable	Tumor/Liver	Tumor/Bone marrow	Tumor/Small intestine	(n)
300 nmole 30 min	90 min	ASF	0.18 $\pm$ 0.04	4.3 $\pm$ 0.4	2.0 $\pm$ 0.3	7
		RNA	2.9 $\pm$ 0.8	18 $\pm$ 7	22 $\pm$ 10	
1200 nmole 30 min	90 min	ASF	0.21 $\pm$ 0.04	7.7 $\pm$ 1.5	4.0 $\pm$ 0.8	7
		RNA	8.1 $\pm$ 2.0	65 $\pm$ 13	79 $\pm$ 27	
24000 nmole 30 min	90 min	ASF	0.07 $\pm$ 0.01	5.2 $\pm$ 1.0	2.9 $\pm$ 0.4	4
		RNA	3.6 $\pm$ 1.1	13 $\pm$ 4	22 $\pm$ 6	
300 nmole 30 min	24 h	ASF	0.36 $\pm$ 0.04	3.6 $\pm$ 0.3	2.3 $\pm$ 0.3	6
		RNA	5.8 $\pm$ 2.7	85 $\pm$ 66	31 $\pm$ 15	
1200 nmole 30 min	24 h	ASF	0.30 $\pm$ 0.02	3.3 $\pm$ 0.3	2.0 $\pm$ 0.2	7
		RNA	3.3 $\pm$ 1.1	17 $\pm$ 8	20 $\pm$ 9	
24000 nmole 30 min	24 h	ASF	0.39 $\pm$ 0.05	3.9 $\pm$ 0.2	1.9 $\pm$ 0.2	6
		RNA	2.4 $\pm$ 0.9	7.8 $\pm$ 3.3	9.2 $\pm$ 3.5	

TABLE V continued

Dose and infusion time	Labelling time	Variable	Tumor/Liver	Tumor/Bone marrow	Tumor/Small intestine	(n)
1200 nmole 2 h	3 h	ASF	0.20 $\pm$ 0.04	11.3 $\pm$ 1.6	3.7 $\pm$ 0.7	9
		RNA	9.8 $\pm$ 2.0	72 $\pm$ 19	50 $\pm$ 11	
300 nmole 2 h	24 h	ASF	0.41 $\pm$ 0.03	3.6 $\pm$ 0.6	2.1 $\pm$ 0.2	7
		RNA	3.1 $\pm$ 1.3	51 $\pm$ 26	18 $\pm$ 7	
1200 nmole 2 h	24 h	ASF	0.32 $\pm$ 0.01	3.2 $\pm$ 0.3	1.9 $\pm$ 0.2	7
		RNA	13 $\pm$ 4	58 $\pm$ 20	66 $\pm$ 31	
24000 nmole 2 h	24 h	ASF	0.33 $\pm$ 0.03	3.6 $\pm$ 0.2	2.0 $\pm$ 0.3	6
		RNA	2.1 $\pm$ 0.8	7.6 $\pm$ 2.8	11 $\pm$ 5	
300 nmole 24 h	24 h	ASF	0.19 $\pm$ 0.05	3.9 $\pm$ 0.3	1.3 $\pm$ 0.5	6
		RNA	3.8 $\pm$ 2.3	4.4 $\pm$ 1.9	5.2 $\pm$ 2.3	
1200 nmole 24 h	24 h	ASF	0.17 $\pm$ 0.02	3.5 $\pm$ 0.3	1.8 $\pm$ 0.2	6
		RNA	4.2 $\pm$ 0.9	20 $\pm$ 5	18 $\pm$ 5	
24000 nmole 24 h	24 h	ASF	0.17 $\pm$ 0.01	3.0 $\pm$ 0.3	1.5 $\pm$ 0.2	6
		RNA	1.4 $\pm$ 0.2	5.0 $\pm$ 1.0	5.6 $\pm$ 1.5	

TABLE VI

Effect of treatment with PALA and D-glucosamine (D-Glc) on the incorporation of ^3H -F-Urd into the ASF, the RNA and the DNA fraction. The Table gives the mean $\pm$ SE. n = number of rats.

Variable	Treatment	Tumor	Liver	Bone marrow	Small intestine	Kidney	n
$\frac{\text{dpm in ASF}}{\mu\text{g DNA}}$	PALA + D-Glc control	226 $\pm$ 80 303 $\pm$ 79	3493 $\pm$ 214 3034 $\pm$ 272	47 $\pm$ 5 42 $\pm$ 4	113 $\pm$ 12 126 $\pm$ 6	1122 $\pm$ 53 ^a 2107 $\pm$ 56	5 4
$\frac{\text{dpm in RNA}}{\mu\text{g DNA}}$	PALA + D-Glc control	56 $\pm$ 27 59 $\pm$ 38	119 $\pm$ 21 ^a 23 $\pm$ 2.5	14 $\pm$ 3 10 $\pm$ 4	20 $\pm$ 1.7 23 $\pm$ 1.8	101 $\pm$ 13 ^b 24 $\pm$ 5	5 4
$\frac{\text{dpm in RNA}}{\mu\text{g DNA}}$	PALA + D-Glc control	0.19 $\pm$ 0.08 0.32 $\pm$ 0.14	2.50 $\pm$ 0.39 ^a 0.82 $\pm$ 0.01	0.48 $\pm$ 0.05 0.57 $\pm$ 0.03	0.12 $\pm$ 0.03 0.20 $\pm$ 0.04	1.25 $\pm$ 0.31 0.41 $\pm$ 0.07	5 4

a = p < 0.001 on the logarithmic values compared to controls.

b = 0.001 < p < 0.01

In paper V was found on days one, three and five a delimited tumor in the liver in all rats. There was more necrosis in the central parts of tumors in rats treated with HAL than in the controls. The tumor has no capsule (Fig V).

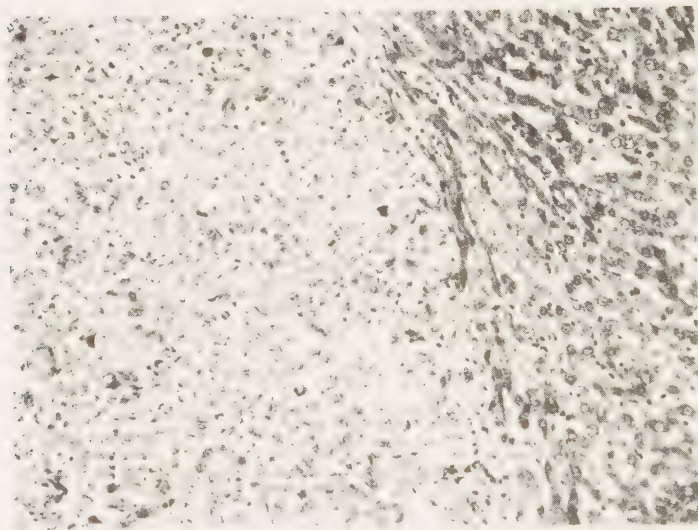


Fig. V Light microscopical section of borderline between tumor and liver tissue 5 days after ligation. The tumor to the left and in the centre is sharply delimited from the liver but there is no capsule. The liver parenchymal cells close to the tumor are compressed. Hematoxylin-erythrosine x 150.

The biochemical examination of the tumors showed a decrease in the content of nucleotides and RNA/g tissue and μg DNA on days one, three and five and in ASF/ μg DNA on days one and three in the tumors in rats treated with HAL.

compared with controls (table VII). No difference was seen between control rats and rats treated with HAL on these days regarding the labelling of the ASF and RNA. In all rats treated with HAL, there was an increase in the amount of RNA/ug DNA ($p < 0.001$) and g tissue ($p < 0.001$) and of nucleotides/g tissue in tumors on day ten compared with day five. The ratio of RNA to ASF labelling was also increased on day ten in all groups compared to HAL rats on day five. The significance was $0.001 < p < 0.01$ between the intraportal groups and $p < 0.001$ compared to the other two administration routes. There was a lower labelling of DNA on day three in HAL rats compared to controls ($0.01 < p < 0.02$). In the liver there was on day five a significantly greater amount of nucleotides and RNA/ug DNA in HAL rats than in controls. The ratio of RNA to ASF labelling after intraportal administration of ^3H -orotic acid was higher on day ten than on day five in HAL rats ($0.001 < p < 0.01$). There were no differences in DNA labelling (table VIII).

The effect of hepatic artery administration of degradable starch microspheres and pyrimidines in paper VI is shown in table IX and X. The incorporation of ^3H -uridine into the ASF of the tumor was significantly increased at three and sixty minutes when given together with microspheres ($p < 0.05$). This was true even for tumor RNA at both three ($p < 0.05$) and sixty minutes ($0.001 < p < 0.01$). In the ASF of the liver a significant reduction of the labelling was seen at three minutes after combined infusion of ^3H -uridine and DSM compared to ^3H -uridine alone. However, at sixty minutes there was no difference. The labelling of liver RNA was neither at three nor at sixty minutes changed by the addition of DSM to ^3H -uridine infusion. Even in the other tissues the combined treatment with ^3H -uridine and DSM did not at any time measured change the incorporation into ASF

TABLE VII

Chemical analysis of tumor. Nt = Nucleotides, ASF = acid-soluble fraction, n = number of animals.

	Group	$\mu\text{g/g}$ tumor		$\mu\text{g}/\mu\text{g}$ DNA		$\text{dpm}/\mu\text{g}$ DNA		$\text{dpm}/\mu\text{g}$ ASF		DNA	RNA/ASF $\times 10^{-3}$	n
		Nt	RNA	DNA	Nt	RNA	DNA	ASF	DNA			
1 day	control	$1.74 \pm .19$	$6.05 \pm .78$	$5.90 \pm .26$	$.29 \pm .03$	$1.01 \pm .11$	372 ± 111	60 ± 23		$.57 \pm .22$	146 ± 14	6
	ligature	$1.12 \pm .11^c$	$2.72 \pm .21^b$	$6.10 \pm .41$	$.19 \pm .02^c$	$.46 \pm .06^b$	381 ± 141	61 ± 29		$.57 \pm .25$	101 ± 31	6
3 days	control	$1.72 \pm .07$	$6.78 \pm .37$	$5.97 \pm .26$	$.29 \pm .01$	$1.13 \pm .03$	176 ± 18	23 ± 5		$.24 \pm .04$	127 ± 16	6
	ligature	$1.03 \pm .07^a$	$2.41 \pm .33^a$	$4.98 \pm .18^c$	$.21 \pm .01^a$	$.48 \pm .01^a$	315 ± 57	31 ± 8		$.06 \pm .03^c$	91 ± 9	6
5 days	control	$1.83 \pm .08$	$6.01 \pm .54$	$6.21 \pm .42$	$.30 \pm .03$	$.97 \pm .07$	144 ± 24	11 ± 3		$.18 \pm .11$	72 ± 3	6
	ligature	$1.17 \pm .08^a$	$2.25 \pm .16^a$	$4.54 \pm .35^c$	$.28 \pm .05$	$.52 \pm .05^a$	306 ± 72^c	22 ± 5		$.10 \pm .05$	69 ± 5	7
10 days	portal	$1.87 \pm .07$	$6.58 \pm .11$	$4.54 \pm .82$	$.48 \pm .10$	$1.70 \pm .34$	335 ± 78	49 ± 17		$.43 \pm .31$	140 ± 21	5
	peritoneal	$2.01 \pm .07$	$7.15 \pm .15$	$6.22 \pm .25$	$.33 \pm .01$	$1.15 \pm .03$	341 ± 91	53 ± 16		$.42 \pm .08$	153 ± 7	5
	femoral	$2.02 \pm .07$	$7.44 \pm .14$	$6.29 \pm .18$	$.32 \pm .02$	$1.19 \pm .04$	486 ± 98	88 ± 23		$.87 \pm .24$	175 ± 10	6

a = $p < 0.001$ compared to corresponding control group.b = $0.001 < p < 0.01$ c = $0.01 < p < 0.02$

TABLE VIII

Chemical analysis of liver*. The same abbreviations as in Table VII.

Group	$\mu\text{g/g liver}$ Nt	$\mu\text{g}/\mu\text{g DNA}$		$\text{dpm} \cdot 10^{-2}/\mu\text{g DNA}$		dpm/dpm RNA/ASF $\cdot 10^{-3}$		n
		RNA	DNA	Nt	RNA	ASF	RNA	
1 day	control	$3.04 \pm .06$	$2.21 \pm .07$	$1.38 \pm .06$	$3.92 \pm .13$	233 ± 50	$.77 \pm .17$	6
	ligature	$3.21 \pm .12$	$2.11 \pm .05$	$1.48 \pm .08$	$4.11 \pm .11$	182 ± 16	$.76 \pm .09$	6
3 days	control	$2.75 \pm .06$	$1.88 \pm .10$	$1.49 \pm .09$	$4.26 \pm .23$	178 ± 19	$.42 \pm .06$	6
	ligature	$2.76 \pm .07$	$1.49 \pm .06$	$1.49 \pm .06$	$4.33 \pm .28$	194 ± 20	$.46 \pm .06$	6
5 days	control	$2.73 \pm .05$	$2.21 \pm .07$	$1.22 \pm .03$	$3.21 \pm .10$	121 ± 16	$.32 \pm .04$	6
	ligature	$2.85 \pm .06$	$2.06 \pm .03$	$1.39 \pm .05^b$	$3.71 \pm .14^c$	171 ± 22	$.36 \pm .04$	7
10 days	portal	$2.83 \pm .17$	$2.09 \pm .03$	$1.36 \pm .10$	$3.80 \pm .32$	146 ± 9	$.42 \pm .07$	5
	ligature	$3.07 \pm .05$	$2.17 \pm .02$	$1.42 \pm .03$	$3.66 \pm .07$	144 ± 14	$.27 \pm .03$	5
	femoral	$2.99 \pm .05$	$2.31 \pm .10$	$1.31 \pm .09$	$3.33 \pm .17$	150 ± 7	$.34 \pm .01$	6

b = 0.001 < p < 0.01 compared to corresponding control group.

c = 0.01 < p < 0.02

TABLE IX

Incorporation of uracil into the ASF and RNA of tissues 3 and 60 minutes after hepatic artery administration of uracil (1.5 mg/100 g BW) alone or mixed with degradable starch microspheres (DSM), 12 mg. Mean values $\pm$ SEM.

	Substances	n	Tumor	Liver	Kidney	Bone marrow
3 MINUTES AFTER INJ.	DPM in ASF ----- $\times 10^{-3}$	5	10.02 $\pm$ 2.13	4.04 $\pm$ 0.36	3.24 $\pm$ 0.24	0.255 $\pm$ 0.010
	μg DNA					
	Uracil					
	Uracil + DSM	6	15.24 $\pm$ 2.44	4.40 $\pm$ 0.56	2.41 $\pm$ 0.17*	0.214 $\pm$ 0.019
	DPM in RNA ----- $\times 10^{-3}$	5	0.021 $\pm$ 0.005	0.010 $\pm$ 0.001	0.006 $\pm$ 0.001	0.0014 $\pm$ 0.0003
	μg DNA	6	0.028 $\pm$ 0.005	0.009 $\pm$ 0.001	0.004 $\pm$ 0.001	0.0012 $\pm$ 0.0002
60 MINUTES AFTER INJ.	DPM in ASF ----- $\times 10^{-3}$	4	4.48 $\pm$ 1.36	4.06 $\pm$ 1.54	0.90 $\pm$ 0.09	0.107 $\pm$ 0.003
	μg DNA	5	7.89 $\pm$ 1.52	4.45 $\pm$ 1.50	0.89 $\pm$ 0.07	0.109 $\pm$ 0.006
	DPM in RNA ----- $\times 10^{-3}$	4	0.013 $\pm$ 0.003	0.036 $\pm$ 0.002	0.007 $\pm$ 0.003	0.0021 $\pm$ 0.0001
	μg DNA	5	0.024 $\pm$ 0.004*	0.039 $\pm$ 0.007	0.007 $\pm$ 0.001	0.0018 $\pm$ 0.0001
	Uracil					
	Uracil + DSM					

* = $p < 0.05$

TABLE X

Incorporation of uridine into the ASF and RNA of tissues 3 and 60 minutes after hepatic artery administration of uridine (1.5 mg/100 g BW) alone or mixed with degradable starch microspheres (DSM), 12 mg. Mean values $\pm$ SEM.

	Substances	n	Tumor	Liver	Kidney	Bone marrow
3 MINUTES AFTER INJ.	DPM in ASF $\times 10^{-3}$	6	4.70 $\pm$ 0.84	7.87 $\pm$ 0.76	2.42 $\pm$ 0.41	0.227 $\pm$ 0.038
	μ g DNA	6	8.73 $\pm$ 1.45*	5.70 $\pm$ 0.47*	1.56 $\pm$ 0.33	0.204 $\pm$ 0.047
	DPM in RNA $\times 10^{-3}$	6	0.013 $\pm$ 0.002	0.025 $\pm$ 0.003	0.014 $\pm$ 0.002	0.015 $\pm$ 0.004
	μ g DNA	6	0.025 $\pm$ 0.003*	0.017 $\pm$ 0.003	0.011 $\pm$ 0.003	0.015 $\pm$ 0.004
60 MINUTES AFTER INJ.	DPM in ASF $\times 10^{-3}$	8	3.86 $\pm$ 0.83	3.23 $\pm$ 0.36	1.34 $\pm$ 0.07	0.137 $\pm$ 0.009
	μ g DNA	11	6.16 $\pm$ 0.59*	3.31 $\pm$ 0.30	1.38 $\pm$ 0.09	0.133 $\pm$ 0.009
	DPM in RNA $\times 10^{-3}$	8	0.025 $\pm$ 0.005	0.073 $\pm$ 0.005	0.057 $\pm$ 0.003	0.022 $\pm$ 0.003
	μ g DNA	11	0.087 $\pm$ 0.020**	0.080 $\pm$ 0.004	0.059 $\pm$ 0.004	0.024 $\pm$ 0.004

* $p < 0.05$ ** $p < 0.01$

or RNA compared to ^3H -uridine treatment alone. Regarding the combination ^3H -uracil and DSM, a significantly increased labelling of tumor RNA was seen at sixty minutes in the rats simultaneously infused with DSM ($0.05 < p < 0.02$). In liver and the other tissues except for the kidney additional DSM had no influence on the incorporation of ^3H -uracil into ASF or RNA. In the kidney a decreased radioactivity of the ASF was seen at three minutes after infusion ($p < 0.05$).

GENERAL DISCUSSION

Administration routes and tissue distribution of pyrimidines and fluoropyrimidines

Since the introduction by Heidelberger and Duschinsky 1957 the cytostatic potential of fluoropyrimidines has been documented in experimental and clinical studies (Heidelberger et al 1983, Kemeny 1983, 1984). In the treatment of colorectal liver metastases these drugs play a major role (Patt et al 1980, Kemeny et al 1985). However, the clinical tumor response rate after intravenous administration is only about 15 to 20 % (Moertel 1978, Kemeny 1983). Using the hepatic artery for infusion the clinical tumor response rate seems to be approximately 50 % in most studies (Patt et al 1980). No convincing effect has been reported in patients with liver metastases from colorectal cancer after intraportal infusion of fluoropyrimidines alone (Taylor 1978). Clinical results are difficult to compare because the criteria used for response and also the stage of disease have not been uniform. Thus it seemed essential to us to compare more exactly the effects of different administration routes using an experimental model which mimics the clinical situation. The predominantly arterial supply to liver metastases has been known since the report of Segall 1923. The importance of the portal system is, however, still maintained (Ackerman 1974, Lin 1984). When the hepatic venous drug concentration of FUra and FdUrd was compared after intravenous and intraarterial administration fivefold higher concentration was found after hepatic artery infusion (Ensminger et al 1978). The intraperitoneal route has been suggested to be as effective as the intraportal (Speyer et al 1981). The importance of the administration route for the incorporation of drugs into a mainly arterially supplied secondary liver tumor is evident from our

results. Using ^3H -uridine as the model substance, the labelling of tumor RNA and ASF was significantly higher by the arterial route compared to all the others. Intravenous administration resulted in an incorporation of the tracer into tumor RNA which was somewhat higher than that achieved by the intraperitoneal or intraportal routes. Thus there is probably a higher ^3H -uridine concentration in the hepatic artery during the first arterial passage after intravenous administration. By intraportal and intraperitoneal infusion, a considerable uptake of ^3H -uridine probably occurs in liver cells at the first passage (Frei III et al 1981, Sadée & Wong 1977, Gasser et al 1981). The tumor uptake by the latter two routes was about equal, in accordance with studies of the drug concentration in the portal system (Speyer et al 1981). The normal liver cells, however, received more drugs by the portal vein infusion.

In several tumor systems the cytotoxicity of fluoropyrimidines seems related to their incorporation into RNA (Kufe & Egan 1981, Glazer & Lloyd 1982). Recently it was recognized that the incorporation into the ASF correlates more closely to cytotoxicity than incorporation into RNA (Meisler & Squeglia 1985). The dose limiting toxicity of fluoropyrimidines appears to be gastrointestinal and hematopoietic in animals as well as in humans (Kirkwood et al 1980, Martin et al 1980, Au et al 1982). A high ratio of fluoropyrimidine incorporation into target molecules of tumor in relation to that of normal tissue should be the aim in our therapeutic efforts. The high ratio between the incorporation of both ^3H -uridine and ^3H -Furd into the RNA of tumor to that of normal tissues confirmed the superiority of the arterial route. Clinical trials using intraarterial fluoropyrimidine infusion, however, have also demonstrated the risk of toxic hepatitis and sclerosing cholangitis after long-term infusion (Kemeny et al 1985). In our

experiments high incorporation into liver RNA was seen after intraarterial and intraportal infusion which might be correlated to clinical liver toxicity.

The clinically most commonly used fluoropyrimidines are FUra and FdUrd. For the treatment of colorectal liver metastases it must be important to select the most proper fluoropyrimidine treatment. Thus it appeared to us essential to investigate the incorporation of different fluoropyrimidines and their natural counterparts into the ASF, RNA and DNA of tumor and normal tissues in the experimental model described. Our results demonstrate that significantly higher incorporation into RNA can be achieved with FdUrd and FUrd than with FUra. However, no obvious differences were seen between FUrd and FUra regarding the ratio of their incorporation into tumor RNA relative to normal tissue RNA. Treatment of human colon carcinoma cells (LoVo) in vitro with FUra showed that all FUra was transformed into FdUrd. Thus the killing effect is likely to be mediated mostly by ribosidation and not by conversion into FdUrd (Treviño et al. 1980). A greater growth delay of human colon adenocarcinoma xenografts in immune deprived mice was seen after FUrd compared with FUra or FdUrd. The incorporation into RNA, however, was after the administration of equimolar amounts of FUrd and FUra similar and only slightly greater than that for FdUrd (Houghton & Houghton 1980). In these experiments the tumor site was subcutaneous and the drugs were given intraperitoneally. However, the differential growth inhibitory effects of the various fluoropyrimidines can be explained by incorporation into different kinds of RNA responsible for the variation in toxicity (Armstrong & Cadman 1983). From experimental studies it is well known that many transplantable neoplasms resistant to either FUra or FdUrd remain sensitive to FUrd (Heideberger et al 1958; Kessel et al 1971). In vitro the human

colocarcinoma cell line HT 29 was approximately a hundred times more sensitive to FdUrd than to FUra (Glazer & Lloyd 1982). In L-1210 cells FUra is converted to FdUrd which is incorporated into RNA but FdUrd is converted to FdUMP which inhibits the thymidylate synthetase (Robool et al 1984). On the other hand, our results showed that FdUrd was incorporated into the RNA and it might be that leukemia cells do not convert FdUrd to FUra. The ratio of incorporation of FdUrd into tumor relative to normal tissue RNA was low which would imply a greater toxicity with this drug. This is in agreement with the findings that the bone marrow toxicity of FdUrd was due to its incorporation into RNA (Diaso et al 1982). In our tumor model the incorporation of the fluoropyrimidines was in general significantly higher than that of the pyrimidines. It has been suggested that uridine nucleotides are located in at least two pools, one of which is rapidly utilized for RNA synthesis while the other is more slowly utilized (Plageman 1971, Shani & Danenberg 1984). Such compartmentalization might explain the different utility of natural and fluorinated pyrimidines, however, even inhibition of the DNA synthesis by fluoropyrimidines has to be considered as an explanation. As there was no obvious difference between FdUrd and FUra in labelling of tumor RNA in relation to normal tissue RNA, we preferred the use of FUra because it was less expensive for the further evaluation of different therapeutic dose schedules.

Prolonged intraarterial infusion of FUra in patients with colorectal liver metastases was recently demonstrated to improve the survival time significantly compared to intermittent high dose treatment by the same route (Ekberg et al 1986). The relationship between the dose of FUra and the length of infusion time appears to be essential for tumor effect and toxicity. Experimentally this

relationship is supported in our model. The incorporation of FURA into RNA did not increase linearly with increasing dosage. Despite of a higher incorporation of FURA into RNA and ASF with increasing doses, the medium dose of 1 200 nmol seemed to be more favourable regarding the ratio between incorporation of FURA into tumor relative to normal tissue RNA. Cytotoxicity is not only a question of FURA uptake into the target molecules of the cells, because fluoropyrimidines are known to inhibit RNA synthesis too (Iapaluti-Espinoza & Franze-Fernandez 1982, Eriksson & Stenram 1984). Thereby the fluoropyrimidines counteract their incorporation into RNA. They also produce a premature break down of false RNA and prevent the formation of ribosomal RNA (Stenram 1966, Stenram & Willén 1970, Stenram et al 1972, Wilkinson et al 1976). Important for fluoropyrimidine cytotoxicity is also the inhibition of DNA synthesis by formation of a thymidylate synthetase-FdUMP- N^5N^{10} -methylene tetrahydrofolate complex which is believed to be found in the DNA fraction (Parker & Klubes 1985). This is in accordance with the results which show a minor incorporation of FURA into this fraction. In tissues with high thymidine kinase activity inhibition of the DNA synthesis requires not only a block of the thymidylate synthesis by FdUMP but also the loss of the salvage pathway i.e. thymidine kinase (Spiegelman et al 1980). Initiation of the DNA synthesis requires the use of a RNA primer molecule (Sawyer et al 1984). Primer molecules containing fluoropyrimidines might not be used by DNA polymerase or removed by the hybridase enzyme. This might be observed as inhibition of DNA synthesis, however, the cause would be fluoropyrimidine RNA rather than a block of thymidylate synthetase by FdUMP (Spiegelman et al 1980). Our results showed that the ASF contained the highest incorporation of FURA and the ratio of FURA incorporation into RNA to that of ASF is recognized to be low for the tumor compared to the liver. This might indicate a decreased utilization for the synthesis of coding RNA in the tumor.

Enhancement of drug incorporation into target molecules

The biochemical rationale for the combination of PALA with fluoropyrimidines is based upon the *in vitro* evidence that PALA enhances the incorporation of FUra into tumor RNA and results in an increased antitumor activity (Kufe & Egan 1981, Ardalan et al 1981, Liang et al 1982). Experimental studies on solid tumors of colon, ovary and lung have shown that the combination of PALA and FUra is clearly more effective than either drug alone (Johnson et al 1980, Spiegelman et al 1980, Ardalan et al 1981). Even if tumor responses are reported in patients with metastatic colorectal carcinoma (Weiss et al 1982) PALA/FUra demonstrate antitumor activity similar to that achieved with FUra alone. PALA used as a single drug was found to have only limited effect against most tumors (Trlichman et al 1979, Valdivieso et al 1980) even if a pronounced inhibition of tumor aspartate transcarbamoylase was observed (Kensler et al 1981, Loo et al 1980). In hepatomas and normal liver the UTP-pool was reduced by the combination of PALA and D-galactosamine (Keppler 1978). Sugar analogues have the property of preventing the circumvention of *de novo* pyrimidine synthesis inhibition by consuming nucleotides supplied by the salvage pathway (reviewed by Keppler & Holstege 1982). There is a cell specificity which is important for the selection of suitable sugar analogues. Hepatocytes and hepatoma cells are target cells for D-galactosamine while TA 3 mammary tumor cells are targets for D-glucosamine (Keppler 1978). In experiments using hepatomas, pretreatment with PALA and D-galactosamine increased the FUrd incorporation into RNA (Holstege et al 1979) and tumor cell lethality was potentiated (Anukarahanonta et al 1980). In a previous experiment with a colon adenocarcinoma in the rat liver, pretreatment with PALA and D-glucosamine potentiated the inhibitory effect of FUrd on tumor growth (Erichsen et al 1982). With this background we

started to determine the effect of such combined treatment on the target molecules of tumor and normal tissues in the described model. In agreement with other studies (Bekesi & Winzler 1969, Keppler et al 1970, Keppler 1977) it was found that PALA and D-glucosamine decreased UTP and increased UDP-N-acetylhexosamine pools in liver and tumor. No effect on the tumor was seen but the liver and the kidney showed increased labelling of RNA. To a large extent pyrimidines are metabolised in the liver (Cooper 1972, reviewed by Keppler & Holstege 1982). So therefore this organ might be more sensitive to interference with the metabolic pathways of these drugs. Most previous studies have been performed on hepatomas in which the metabolism might be different from that of adenocarcinomas. In human colorectal adenocarcinomas the activity of uridine kinase was found to be very low compared to that of other neoplastic tissues which might be an explanation (Ahmed et al 1981). In patients with breast cancer treated with FUra, differences was seen between the responders and non-responders with respect to the in vitro conversion rates of FUra to FUrd but not to FdUrd or FUMP. Differences were also seen in the generation rate of triphosphates; in tumor samples of responders the diphosphate kinase was significantly increased (Ardalan et al 1981). The formation and accumulation of phosphorylated derivatives of D-glucosamine are associated with an enhanced catabolism of adenine nucleotides (Bekesi & Winzler 1969, Holstege et al 1982). This might influence the synthesis of fluoropyrimidine containing RNA as ATP is an important energy source. Glucose competitively inhibits the transport of glucosamine into the cell and also enhances the capacity of the cell to produce ATP (Plageman & Erbe 1973). Differences in glucose content between liver cells and adenocarcinoma cells might play a possible role. This underlines the desirability of better knowledge of the biochemistry of tumors considered

suitable for fluoropyrimidine treatment. The increased labelling of kidney RNA seen after pretreatment with PALA and D-glucosamine might reflect the high urinary excretion of PALA. Probably this implies an uptake of PALA in the kidney cells resulting in increased utilization of FUrD for RNA synthesis. It seems that the previously reported increased inhibitory effect of FUrD on the growth of this tumor in rats pretreated with PALA and D-glucosamine (Erichsen et al 1982) cannot be related to enhanced incorporation of FUrD into tumor cell RNA. PALA and D-glucosamine might, however, contribute to the cytotoxic effects of FUrD by other mechanisms such as interference with RNA, DNA and cell membrane synthesis (Friedman & Skehan 1980, Moyer et al 1982, Chelibonova-Lorer et al 1983).

There is general agreement that liver metastases are mainly supplied by the hepatic artery but disagreement still persists about the importance of the portal blood supply (Seqall 1923, Breedis & Young 1954, Honjo & Matsumura 1965, Nilsson & Zettergren 1967, Ackermann 1974, Carlsson et al 1981, Lin 1984). In the tumor periphery the possibility of blood supply from both the arterial and portal vessels has to be considered (Ackermann 1974, Lin 1984).

Treatment of liver metastases from colorectal cancer by intraportal infusion of fluoropyrimidines was found to increase survival in patients treated with a combination of hepatic artery ligation and intraportal FUra infusion. No benefit could be recognized in patients with perfusion by the portal vein alone when compared to a non-treated control group (Taylor 1978). Hepatic artery ligation was first suggested by Markowitz 1952 and has been widely used for the treatment of liver tumors but with limited and only temporary clinical effect

(Fortner et al 1973, Lee Yen 1983). The shortlived effect of HAL alone can be explained by the development of new arterial collaterals in both tumor and normal liver after a few days (Redman & Renter 1970, Bengmark & Rosengren 1970, Stridbeck et al 1984). It has been postulated that following HAL a compensatory increased blood flow in the portal vein occurs. Increased portal blood flow to the tumor periphery and possibly a portal revascularization after HAL might result in high drug exposure to surviving tumor cells following drug infusion through the portal vein. In vitro experiments on isolated nuclei from liver cells recovering from reversible non-necrogenic ischemia has shown that RNA synthesis recovers with an overshooting well above normal and lasting at least for twenty-four hours (Schiaffonati et al 1978). This situation could be expected in the tumor periphery after HAL and tumor cells in this area might be more susceptible during the recovery phase to drugs like the fluoropyrimidines. Orotic acid is an intermediate metabolite in pyrimidine synthesis and is widely used as an RNA precursor. As shown in table III its uptake and incorporation into RNA and DNA indicate how fluoropyrimidines might be taken up. Microscopically the tumor at different time intervals showed more tumor necrosis in rats treated with HAL than in controls. On day ten the HAL -rats still had limited tumor while the control rats had widespread tumor growth in the liver and the peritoneum. This is consistent with previous experimental studies showing regression of tumor and significant prolongation of survival after HAL alone (Carlsson 1982, Hafström & Carlsson 1984). Xenon blood flow studies in liver tumors showed a 90 % decrease in tumor and 35 % decrease in normal liver perfusion after HAL (Gelin et al 1968, Taylor et al 1979). After HAL there was a decrease in tumor nucleotides and RNA but no such decrease was evident in the liver. This seems to reflect the dual vascular supply of the liver and the

predominantly arterial supply to the tumor which makes the latter sensitive to HAL. At day ten after HAL the nucleotide and RNA contents of the tumor had reached the same values as the control rats at day one and five. At this time the RNA content of HAL rats was significantly increased compared to HAL rats sacrificed after five days. This indicated a recovery of the tumor viability probably due to revascularization which could be either of arterial or portal origin. The labelling experiments showed lower labelling of tumor DNA in rats subjected to HAL than in controls on day three and a trend to lower labelling of tumor RNA on days three and five in both groups. In the control rats this might be explained by progressive tumor growth with less accessibility of portal vein blood flow to the tumor, and in the HAL rats perhaps also by a decreased viability. The results do not support the idea of an increased tumor vascularization from the portal vein at day three and five. The labelling of tumor RNA at day ten was the same as on day one in the HAL rats, which supports the hypothesis of tumor revascularization. If this were of portal origin, however, a higher labelling of tumor RNA and ASF could have been expected after intraportal rather than after the femoral vein infusion. Therefore in spite of the fact that portal vessels have been found in the periphery of, and some also reaching the central part of the tumor (Carlsson 1982), they do not seem to have any therapeutic relevance. Consequently portal infusion of cytostatic agents can not be more effective than systemic administration when given a week or more after HAL. But repeated transient dearterialization was recently shown in pigs to prevent collateral vessel formation (Persson et al 1986). It was also shown that a combination of transient liver ischemia and intraarterial infusion of FUra is well tolerated (Persson et al 1985). These findings might have importance for the treatment of liver tumors and should be tested in a tumor model which permits transient ischemia.

In all cytostatic treatment our intention is to get as much as possible of the agent into the target molecules of the tumor cells and as little as possible into those of the normal tissues. For tumors in the liver, regional infusion through the hepatic artery is superior to systemic administration. In previous studies it was shown that drugs might remain in an organ when administered intraarterially together with DSM (Lindell et al 1978, Tuma et al 1982). A concomitant reduced systemic exposure was also seen by some (Dakhil et al 1982, Gyves et al 1983) but not by others (Teder et al 1985). Repeated administration of DSM into the hepatic artery of tumor-free rats has been performed without any adverse effects to the liver cells (Lindell 1977). In a more recent study DSM were injected through the same route concomitant with a nucleic acid precursor. The microspheres neither affected liver cell energy nor the incorporation of the nucleic acid precursor into RNA of the liver cell (Teder et al 1985). The reason for this must be the mainly portal supply of blood to the liver which will protect it from ischemic effects resulting from a reduced arterial blood flow by the DSM. Infusion of DSM into the hepatic artery of rats with a liver tumor showed a proportionally greater reduction in the blood flow to tumor than to normal liver (Lindell 1977). As liver tumors are mainly nourished by the hepatic artery they will be more sensitive to reduced arterial blood flow caused by the DSM. This vascular situation might promote the drug uptake into tumor. Therefore it is of utmost importance to decisively determine how DSM influence the incorporation of a pyrimidine base and nucleoside into the target molecules of tumor and normal tissues. The two nucleic acid precursors might use different transport systems across the cell membrane (Cohen et al 1979), as well as different enzyme systems within the cell (reviewed by Keppler & Holstege 1982, Shani & Danenberg 1984). For future treatment it is valuable to find the best

metabolite for combined treatment with DSM. Our results demonstrate a significantly enhanced incorporation of ^3H -uridine and ^3H -uracil into RNA of the tumor 60 minutes after concomitant infusion with DSM. No differences of RNA labelling were seen in the normal tissues after combined infusion or drug infusion alone. This is in accordance with the results in previous studies with ^{14}C -thymidine in rats without tumors (Teder et al 1985). The tolerance of rats to high doses of FUra injected by the hepatic artery with and without DSM was investigated by Lindell, who showed that the combination with microspheres increased survival compared to FUra infusion alone (Lindell et al 1978). DSM and FUra was also studied in a clinical trial (Aronsen et al 1979), however, the number of patients was small and the liver tumors were of different origin and therefore no definite conclusions can be drawn. Further studies with therapeutic doses of fluoropyrimidine and DSM seem to be of great importance.

Future aspects

Further exploration of the metabolic pathways of pyrimidines and fluoropyrimidines are essential for detection of mechanisms, which might be targets for synergistic drug combination. Interesting aspects of this have been demonstrated in studies investigating co-administration of fluoropyrimidines with thymidine, citrovorum factor, methotrexate, 6-methylmercaptopurine, allopurinol and pyrimidines and purines (Spiegelmann et al 1980, Sawyer & Stolfi 1981, Mihich & Rustum 1985, Houghton et al 1982, Kufe & Egan 1981, Clark & Slevin 1985, Iigo & Hoshi 1984, Lockshin et al 1985). These compounds showed different modes of interference with the metabolism of pyrimidines. Thus intraarterial administration of cytotoxic drugs with simultaneous intravenous infusion of an agent with potential protective properties seems attractive and possible. It is important to find the optimal fluoropyrimidine metabolite and therefore mandatory to develop technics which are capable of identifying the enzyme patterns of tumors. A more selective manipulation of the fluoropyrimidine metabolism might thereby be possible. Biopsies from human tumors and transplantation into nude rats might give further opportunity to develop suitable treatment schedules. The nutrition state of cancer patients might influence the therapeutic response to chemotherapy and therefore further investigations regarding the effects of different diets on the incorporation of various fluoropyrimidines into the target molecules of tumor and normal tissues must be performed. In the treatment of liver metastases from colorectal cancer the dual blood supply of the liver and the predominantly arterial blood supply of the tumor make further investigation to find methods which permit the lasting ischemia of the tumor but without influence on the liver important. Tumor cells and especially hypoxic cells are sensitive to hyperthermia (Overgaard 1977, Pheasant

et al 1984). A combination of DSM and local hyperthermia was shown to produce significantly reduced tumor growth compared to local hyperthermia alone in this model (Erichsen et al 1985). It therefore appears to be of interest to investigate a sequential treatment schedule including fluoropyrimidines to find optimal intervals between hypoxic-hyperthermic treatment and chemotherapy. Even combination with local irradiation might be of interest and intraarterial administration of radioactive isotopes can be performed (Ariel and Padula 1978, Grady 1979). However, theoretically more speculative would be the use of DSM with degradation properties correlated to degradation of radioactive compounds incorporated into the microspheres or loading of microspheres with radioactive tumor cell antibodies which might increase tumor selectivity. Important effects of irradiation as well as cytostatics are the formation of free radicals which attack the purine and pyrimidine nucleotides in RNA and DNA and even other important macromolecules of cytosol and cell membranes. This opens further perspectives for future cancer therapy.

CONCLUSION

The following conclusions can be drawn from the experimental work presented in this thesis.

- I Hepatic artery administration of pyrimidines exemplified by ^3H -uridine is superior to portal or systemic administration with a significantly higher incorporation of the metabolite into the target molecules of tumor but significantly lower incorporation into that of the normal tissues.

Hepatic artery administration of the fluoropyrimidine ^3H -FUrd revealed a similar ratio between incorporation into the target molecules of tumor to normal tissues as that which was demonstrated for ^3H -uridine. For the treatment of a liver tumor with fluoropyrimidines this indicates a favourable therapeutic index when the hepatic artery is used for the drug administration.

- II Administration of various pyrimidines and fluoropyrimidines by the hepatic artery revealed that the fluorocompounds were incorporated into RNA of the tumor in significantly greater amounts than the normal counterparts. Furthermore it was demonstrated that the fluorinated pyrimidine nucleosides (FUrd, FdUrd) were incorporated into the target molecules of tumor to a significantly higher degree than the corresponding base (FUra).

- III With increasing dosages of FUra administered via the hepatic artery the incorporation of the drug into the target molecules was also increased. However, the ratio between incorporation into RNA of tumor to that of normal tissues was definitely higher by using an intermediate FUra dosage and infusion time.
- IV Hepatic artery administration of the antipyrimidine PALA and D-glucosamine depressed the UTP pool of both the tumor and the liver. No effect of these drugs was recognized on the incorporation of FUrd into the target molecules of the tumor but the RNA labelling of the liver was increased five-fold. This indicates an enhanced liver toxicity when antipyrimidines are combined with fluoropyrimidines.
- V Hepatic artery ligation caused more necrosis in central part of the tumor compared to controls. A significant reduction of tumor nucleotides and RNA content was demonstrated three and five days after HAL but no effect was seen on that of the liver.

Furthermore a decreased incorporation into nucleic acids of intraportally infused ^3H -orotic acid was demonstrated three and five days after HAL. This does not support the hypothesis of portal revascularization or increased pyrimidine anabolism after HAL.

Ten days after HAL a recovery of RNA labelling of tumor was recognized. This indicates an arterial revascularization of the tumor, because at this time intraportal infusion of ^3H -orotic acid was not more effective than systemic administration regarding the incorporation into the target molecules.

VI Administration of DSM by the hepatic artery in combination with a pyrimidine base and a pyrimidine nucleoside showed significantly enhanced incorporation of both metabolites into RNA of the tumor compared to administration of either metabolite without DSM.

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EFFECTS OF ADMINISTRATION ROUTES ON THE UPTAKE OF URIDINE AND 5 - FLUOROURIDINE INTO AN ADENOCARCINOMA TRANSPLANTED TO RAT LIVER

Christian Erichsen, MD¹, Per-Inge Christenson, MD², Göran Eriksson, Ph.D.², Torsten Yngner, Ph.D.¹, Per-Ebbe Jönsson, MD¹, Unne Stenram, MD²

Departments of Surgery¹ and Pathology², University of Lund, S-221 85 Lund, Sweden.

Correspondence to: Per-Ebbe Jönsson, Department of Surgery, University of Lund, S-221 85 Lund, Sweden

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Key words: ^3H -uridine, ^3H -5-fluorouridine, secondary liver tumor, administration routes

ABSTRACT

In a model of secondary liver cancer in Wistar rats the effect of different administration routes on the uptake of ^3H -uridine into tumor and several normal tissues was studied. The rats were inoculated with a tumor cell suspension in the central liver lobe. Ten days later they were distributed into four groups with a catheter placed in the gastroduodenal artery, the portal vein, one of the femoral veins or in the peritoneal cavity. ^3H -uridine was injected 46 hours later and after further 90 minutes the animals were anaesthetized and pieces of liver tumor and normal tissues were removed and frozen. The incorporation into the acid soluble fraction and RNA was analysed. In a separate experiment ^3H -fluorouridine was administered by the gastroduodenal artery and a comparison was made with the uptake of ^3H -uridine. A significantly higher amount of uridine was incorporated into the tumor by the arterial route. The intraportal and intraperitoneal routes were comparable while a somewhat higher incorporation was found by the systemic route. The consequence of using the different routes upon the incorporation into RNA of tumor and dose limiting organs are demonstrated. With the aid of this experimental model it is possible to further evaluate the effect by different manipulations on different drugs regarding the administration route.

INTRODUCTION

The dual vascular supply of the liver and colorectal liver metastases raises the question of how to use the vascular systems in the therapy. In clinical practice the hepatic artery is most frequently employed (1). This is based on a vast number of studies regarding liver tumor circulation, however a recent study maintains the importance of the portal system in nourishing the metastases (2, 3). When the hepatic venous drug concentrations of 5-fluorouracil (5-FU) or 5-fluorodeoxyuridine (5-FdUrd) are analysed and compared after intravenous and intraarterial infusion an up to five fold higher concentration is found by the hepatic artery infusion (4). The intraperitoneal route is suggested to be as effective as the intraportal and the accessibility is easier. The rationale is that the portal venous concentration of 5-FU is equal almost after intraperitoneal and intraportal administration (5).

With the aid of the present experimental model, which mimics the clinical situation, it is possible to compare more exactly the influence of different administration routes. The optimum should be a selective uptake of the cytotoxic drug by tumor cells and minimal by normal tissues. The aim of this study was to explore the effects of different administration routes on the incorporation of ^3H -uridine (^3H -Urd) and ^3H -5-fluorouridine (^3H -5-FUrd) into a transplantable adenocarcinoma of the liver compared with normal tissues.

MATERIAL AND METHODS

Animals and tumors:

Inbred female rats of the Wistar strain weighing 145 - 210 g were used for the experiments. The animals were kept individually and given access to standard laboratory diet and tap water ad libitum. In all animals 1.0×10^6 cells from an N-methyl-N-nitrosoguanidine induced adenocarcinoma of the colon (6) were inoculated into the central liver lobe under ether anesthesia.

Chemicals:

5, 6- ^3H -uridine (38.9 Ci/mmol) and 5, 6- ^3H -fluorouridine (16.5 Ci/mmol) were purchased from New England Nuclear Corporation (Boston, Ma). ^3H -FUrd was delivered in 70 % ethanol and therefore freeze dried overnight and dissolved in sterile water immediately before use. All chemicals used were pro analysi.

Experimental design:

At laparotomy 10 days after the cell inoculation, a catheter (Intramedic PE 10; Clay Adams, Parsippany, N.J.) was placed into the gastroduodenal artery, the portal vein, a femoral vein or into the peritoneal cavity. At this time the solitary tumor had a volume of approximately 0.25 cm^3 when calculated according to the formula $V = a \cdot b^2/2$, where A was the

largest and B the smallest diameter (7). The animals were placed in individual cages with a catheter connected to a pump (Injectomat R50, Fresenius, Bad Homburg FRG) which infused 5 ml 0.9 % NaCl solution/24 h. All tracers were given through the catheter and the infusion time was 15 seconds. The catheters were flushed after each infusion by 0.5 ml 0.9 % NaCl solution during 15 seconds.

Thirteen animals were used to investigate the effect of different administration routes on the incorporation of uridine into the acid soluble fraction and RNA of tumor and normal tissues. ^3H -uridine (1250 microCi/1000 g BW) was injected 36 h after the laparotomy (at about 9 a.m.). 90 minutes later the animals were anaesthetized in a diethylether-oxygen atmosphere. In consecutive order liver, tumor, right kidney, spleen, a piece of small intestine, thymus and femoral bone marrow were removed and immersed in liquid nitrogen.

The same procedure was used to study 5 animals given ^3H -FURd (1250 microCi/1000g BW) intraarterially.

Chemical analysis:

The acid soluble fraction and RNA were extracted according to Munro and Fleck (8). The radioactivity was measured in a Packard (Downers Grove Ill.) Tri Carb CD 300 liquid scintillation system. The acid soluble fraction contains all compounds soluble in 0.1 Mperchloric acid. This fraction and RNA were calculated as microgram of ultraviolet absorbing substances.

DNA was analysed by the technique of Scott et al (9) as modified by Hinrichs et al. (10).

Statistical analysis:

Students' two-tailed t-test was employed on the logarithmic values.

RESULTS

The influence of different administration routes on the incorporation of ^3H -uridine into the tumor acid soluble fraction and RNA is shown in Table I. A more than twofold higher incorporation into the tumor acid soluble fraction was demonstrated by the intraarterial administration compared to the others. The difference in specific radioactivity of RNA was even more pronounced with a tenfold higher radioactivity of RNA by the hepatic artery compared to systemic administration and sixtyfold higher when compared to the intraperitoneal route. In the liver the labelling of the acid soluble fraction was almost equal and high by all four administration routes (Table I). Concerning liver RNA the intraarterial and the portal vein infusions resulted in high radioactivity whereas low was registered after the intraperitoneal and femoral vein infusions. The specific radioactivity of RNA in peripheral normal tissues, e.g. bone marrow and small intestine, was low after infusion by the hepatic artery and the portal vein. In the spleen there was a very high incorporation of ^3H -uridine into RNA after intraarterial administration. The femoral vein infusion resulted in high incorporation into the acid soluble fraction of

both bone marrow and kidney. The labelling of tumor RNA in relation to other organs is shown in Table II. ^3H -FUrđ gave results very similar to those obtained with ^3H -Urđ (Table II and III).

DISCUSSION

The experimental model used is well established and the tumor vascular supply emanates primarily from the hepatic artery (11). Our results clearly demonstrate the importance of the administration routes in the incorporation of drugs into a mainly arterially supplied secondary liver tumor. The specific radioactivity of tumor RNA was significantly higher by the arterial route compared with all the others (Table I). The systemic administration resulted in a somewhat heavier labelling of the tumor RNA than achieved by the intraperitoneal or intraportal, probably because of higher uridine concentration in the artery during the first recirculation after infusion. By the two latter routes there was probably a considerable uptake of uridine by liver cells at the first passage. The tumor uptake by those two routes were the same in accordance with studies of the drug concentration in the portal system (15). As expected the normal liver cells received more drugs by the portal vein infusion.

In the other organs there was a surprisingly high uptake in the spleen after intraarterial administration which could be explained by technical failures at infusion and a spill-over into the splenic artery and no metabolism of the drug in the spleen (12). The high labelling in bone marrow and kidney after the femoral vein infusion reflects the high arterial flow into and

appreciable extraction of drug by these organs. By intraperitoneal infusion a high labelling of the intestine was obtained that could be explained by absorption through the intestinal wall. Following intraperitoneal administration the labelling in liver was lower and in bone marrow and kidney higher than after infusion by the portal vein indicating a substantial uptake of the labelled substance into the systemic circulation rather than into the portal.

Different fluoropyrimidines, mainly 5-FU are commonly used in the treatment of colorectal liver secondaries. In several tumor systems the cytotoxicity of these compounds seems to be related to their incorporation into RNA (13, 14). A dose limiting toxicity appears to be mainly gastrointestinal and hematopoietic in mouse as well as in man (15, 16, 17). A main goal must therefore be to achieve a high ratio of incorporation into tumor RNA in relation to normal tissue. In Table II the ratio is given between the uptake into RNA of tumor and the different organs in relation to the administration route. Of interest, except the superiority of arterial route, is the similarity between intraportal, intraperitoneal and systemic administration. ^3H -uridine and ^3H -FUrd disclosed very similar patterns (Table III) in agree with their similar anabolism (18).

Thus, the present results support the advantage of using the hepatic artery for infusion of 5-FUrd into the liver with metastases because of the high availability of the drug to the tumor and the low host toxicity. The present model is very suitable for further studies of the incorporation of different 5-fluoropyrimidines and also of other anticancer drugs into tumor and normal tissues.

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Table 1. Experiment 1. Effect of administration route on the incorporation of ³H-uridine into the acid-soluble fraction (ASF) and RNA of tumor and normal tissues. s = specific activity. The Table gives the means $\pm$ S.E. Number of rats is given in Table 2.

	Route of administration	Tumor	Liver	Bone marrow	Kidney	Small intestine	Spleen	Thymus
sASF	Gastrooduodenal art	5048 $\pm$ 1700	1808 $\pm$ 460	808 $\pm$ 186	1084 $\pm$ 343	601 $\pm$ 158	1205 $\pm$ 251	1188 $\pm$ 438
	Portal vein	1624 $\pm$ 330	1723 $\pm$ 77	1060 $\pm$ 85	1279 $\pm$ 61	798 $\pm$ 22	1244 $\pm$ 51	862 $\pm$ 86
	Intraperitoneal	1449 $\pm$ 89	1191 $\pm$ 88	907 $\pm$ 45	1035 $\pm$ 79	1266 $\pm$ 195	1058 $\pm$ 74	1151 $\pm$ 20
	Femoral vein	1541 $\pm$ 17	1369 $\pm$ 87	1168 $\pm$ 101	1679 $\pm$ 133	1146 $\pm$ 133	1070 $\pm$ 7	1267 $\pm$ 38
sRNA	Gastrooduodenal art	584 $\pm$ 211	65 $\pm$ 21	12.7 $\pm$ 5.6	19.3 $\pm$ 9.2	15.4 $\pm$ 5.0	84 $\pm$ 30	5.2 $\pm$ 1.5
	Portal vein	9.18 $\pm$ 2.2 ^a	79 $\pm$ 8.2	11.0 $\pm$ 1.0	14.7 $\pm$ 0.9	14.1 $\pm$ 0.8	8.1 $\pm$ 0.6 ^b	4.8 $\pm$ 0.7
	Intraperitoneal	7.4 $\pm$ 0.8 ^a	24 $\pm$ 5.1	4.3 $\pm$ 3.4	30 $\pm$ 13.7	74 $\pm$ 25	8.6 $\pm$ 1.0	14.6 $\pm$ 2.1 ^c
	Femoral vein	43 $\pm$ 0.2 ^b	30 $\pm$ 0.3	101 $\pm$ 15.9 ^b	142 $\pm$ 23	63 $\pm$ 18.1	27 $\pm$ 1.1	27 $\pm$ 3.5 ^b
dpm in ASF/ μ gDNA	Gastrooduodenal art	1728 $\pm$ 220	2203 $\pm$ 591	154 $\pm$ 27	774 $\pm$ 226	243 $\pm$ 61	196 $\pm$ 39	294 $\pm$ 103
	Portal vein	697 $\pm$ 122 ^c	2067 $\pm$ 34	187 $\pm$ 5	880 $\pm$ 31	343 $\pm$ 30	246 $\pm$ 22	136 $\pm$ 11
	Intraperitoneal	529 $\pm$ 36 ^a	1723 $\pm$ 163	190 $\pm$ 32	777 $\pm$ 45	400 $\pm$ 67	238 $\pm$ 23	186 $\pm$ 15
	Femoral vein	619 $\pm$ 45 ^b	1914 $\pm$ 136	240 $\pm$ 30	1228 $\pm$ 80	390 $\pm$ 11	263 $\pm$ 25	219 $\pm$ 6
dpm in RNA/ μ gDNA	Gastrooduodenal art	612 $\pm$ 95	253 $\pm$ 76	7.2 $\pm$ 2.9	19.4 $\pm$ 10.2	11.8 $\pm$ 3.8	41 $\pm$ 15.9	2.2 $\pm$ 0.9
	Portal vein	15.2 $\pm$ 5.7 ^a	280 $\pm$ 34	6.8 $\pm$ 0.8	15.0 $\pm$ 1.1	10.5 $\pm$ 0.3	4.5 $\pm$ 0.5 ^b	1.2 $\pm$ 0.2
	Intraperitoneal	10.1 $\pm$ 1.7 ^a	100 $\pm$ 22	32 $\pm$ 7	30 $\pm$ 12.1	56 $\pm$ 17.4	4.9 $\pm$ 0.6 ^b	5.3 $\pm$ 0.8
	Femoral vein	60 $\pm$ 1.4 ^a	124 $\pm$ 5	63 $\pm$ 10.7 ^b	158 $\pm$ 24	48 $\pm$ 12.9	16.1 $\pm$ 1.0	10.1 $\pm$ 0.9 ^c
ASF/DNA	All routes	0.40 $\pm$ 0.03	1.33 $\pm$ 0.04	0.20 $\pm$ 0.001	0.72 $\pm$ 0.001	0.39 $\pm$ 0.01	0.21 $\pm$ 0.01	0.19 $\pm$ 0.02
	RNA/DNA	1.35 $\pm$ 0.08	3.96 $\pm$ 0.09	0.62 $\pm$ 0.02	1.02 $\pm$ 0.02	0.77 $\pm$ 0.02	0.55 $\pm$ 0.02	0.35 $\pm$ 0.02

a = p < 0.001
b = 0.001 < p < 0.01
c = 0.01 < p < 0.05
compared to arterial administration

Table 2. Experiments 1 and 2. Ratio between dpm in RNA/ μ g DNA in tumor to each of the other tissues. The table gives the means $\pm$ S.F.

Route	^3H -uridine				^3H -Furd
	Gastroduodenal artery	Portal vein	Intraperitoneal	femoral vein	Gastroduodenal artery
<u>Tumor Liver</u>	1.6 $\pm$ 0.4	0.1 $\pm$ 0.02 ^b	0.1 $\pm$ 0.01 ^a	0.5 $\pm$ 0.03 ^c	3.2 $\pm$ 0.7
<u>Tumor Bone marrow</u>	66.1 $\pm$ 24.1	2.5 $\pm$ 1.3 ^b	1.7 $\pm$ 0.2 ^a	1.1 $\pm$ 0.2 ^a	89.7 $\pm$ 14.9
<u>Tumor Small intestine</u>	38.3 $\pm$ 14.6	1.4 $\pm$ 0.5 ^b	0.3 $\pm$ 0.1 ^a	1.5 $\pm$ 0.4 ^b	87.7 $\pm$ 20.7
<u>Tumor Kidney</u>	30.2 $\pm$ 13.2	1.1 $\pm$ 0.5 ^c	0.5 $\pm$ 0.2 ^b	0.4 $\pm$ 0.1 ^b	20.0 $\pm$ 3.4
<u>Tumor Spleen</u>	11.3 $\pm$ 3.4	3.7 $\pm$ 1.7	2.3 $\pm$ 0.7	3.8 $\pm$ 0.2	24.3 $\pm$ 3.6
<u>Tumor Thymus</u>	215 $\pm$ 71.9	13.3 $\pm$ 5.4 ^b	1.9 $\pm$ 0.4 ^a	6.0 $\pm$ 0.5 ^a	1480 $\pm$ 447 ^b
number of animals	3	3	4	3	5

a = $p < 0.001$

b = 0.001 $< p < 0.01$

c = 0.01 $< p < 0.02$

compared to the group given ^3H -uridine by the arterial route.

Table 3. Experiment 2. Incorporation of ^3H -FUrđ into acid-soluble fraction and RNA.
The table gives the means $\pm$ S.F.

	Tumor	Liver	Bone marrow	Small intestine	Kidney	Spleen	Thymus
$\frac{\text{dpm in ASF}}{\mu\text{gDNA}}$	853 $\pm$ 121	5089 $\pm$ 381	59 $\pm$ 2.8	148 $\pm$ 12	2610 $\pm$ 235	106 $\pm$ 6.6	55 $\pm$ 6.1
$\frac{\text{dpm in RNA}}{\mu\text{gDNA}}$	302 $\pm$ 35	110 $\pm$ 18	3.6 $\pm$ 0.6	4.7 $\pm$ 1.7	16 $\pm$ 2.0	2.7 $\pm$ 0.6	0.3 $\pm$ 0.05

Incorporation of Pyrimidines and 5-Fluoropyrimidines into Normal Tissues and an Adenocarcinoma Transplanted into the Liver in Rat*

Per Inge Christensson¹, Christian Erichsen², Per-Ebbe Jönsson², and Unne Stenram¹

¹ Departments of Pathology and

² Surgery, University of Lund, S-221 85 Lund Sweden

Summary. In a model of secondary liver cancer in Wistar rats, the incorporation of tracer doses of pyrimidines and 5-fluoropyrimidines into the acid-soluble fraction, RNA and DNA of several normal tissues and of an experimental adenocarcinoma of the colon transplanted to the liver of rat was determined 90 min after infusion of the substances via the gastroduodenal artery. There was a higher incorporation after injection of FUra than after uracil into tumor RNA. There was very little labeling of DNA by FdUrd but a greater labeling of RNA following injection of this substance than following deoxyuridine in all tissues including tumor except in liver, where it was of the same magnitude. All fluoro compounds gave high labeling of the acid-soluble fraction of the kidney and liver. The experiments will be continued with therapeutic doses of the fluoro compounds.

Key words: 5-Fluoropyrimidines · RNA · Liver metastases · Rats

Introduction

5-Fluoropyrimidines are the most commonly used cytostatic agents in the treatment of liver metastases from colorectal carcinoma (Gustavsson et al. 1981). Very little is known, however, about the extent to which these compounds are taken up by tumor and by normal tissues and incorporated into target sub-

stances, factors of great significance with regard to tumor response and host toxicity.

To study these questions and to mimic the clinical situation, an N-methyl-N-nitrosoguanidine-induced transplantable adenocarcinoma of the rat colon was inoculated into the liver. The uptake of ³H-uridine into the acid-soluble fraction and RNA of the tumor was much higher when given via the gastroduodenal artery compared to the portal vein, a femoral vein or i.p. Uptake was low in several normal tissues. A pilot experiment disclosed similar behavior of ³H-FUrd, administered via the gastroduodenal artery (Erichsen et al. 1985). In order to find the most suitable 5-fluoropyrimidine for treatment of colorectal liver metastases, it was considered of interest to examine how 5-fluoropyrimidines and their natural counterparts are extracted by tumor and normal tissues and incorporated into RNA and DNA in this experimental tumor system. In the present study this was examined after administration of the substances in tracer amounts via the gastroduodenal artery as a basis for studies with therapeutic doses. The labeling time chosen was 90 min because it gave good labeling of DNA and nuclear RNA and low but discernible labeling of ribosomal and other cytoplasmic RNA.

Materials and Methods

Animals and Tumors. Inbred female rats of the Wistar FU strain (Anticimex, Stockholm, Sweden) weighing 145-210 g were used in the experiments. The rats were kept individually and were given access to standard laboratory diet and tap water ad libitum. An N-methyl-N-nitrosoguanidine-induced transplantable adenocarcinoma of rat colon was used (Steele and Sjogren 1974). Under ether anesthesia a cell suspension containing 1.0×10^6 tumor cells in 0.1 ml 0.9% NaCl was injected into the central liver lobe through a midline incision.

Chemicals. 5,6-³H-uracil (42 Ci/mmol), 5,6-³H-FUra (16.5 Ci/mmol), 5,6-³H-uridine (38.9 Ci/mmol), 5,6-³H-FUrd (17.4 Ci/mmol), 6-³H-deoxyuridine (18.0 Ci/mmol), 5,6-³H-FdUrd (14.7 Ci/mmol) and 5-³H-urotic acid (20 Ci/mmol) were purchased from New

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Offprint requests to: Per Inge Christensson, Department of Pathology, University Hospital of Lund, S-221 85 Lund, Sweden.

Abbreviations: FUra, fluorouracil; FUrd, fluorouridine; FdUrd, fluorodeoxyuridine.

England Nuclear Corporation (Boston, Mass.). The fluoro-substances were delivered in 70% ethanol. They were freeze-dried overnight and dissolved in sterile distilled water before use. All chemicals used were pro analysi.

Experimental Design. At laparotomy 10 days after tumor inoculation, a catheter (Intramedic PE 10, Clay Adams, Parsippany, NJ) was placed into the gastroduodenal artery. At this time the tumor volume was about 0.25 cm^3 , when calculated according to the formula $V = a \times b^2 / 2$, where a is the largest and b the smallest diameter (Carlsson et al. 1983). The animals were placed in individual cages with the catheter connected to an infusion pump (type 1850, Braun AG, FRG), giving 1 ml 0.9% NaCl solution per 24 h. On the following day between 8 a.m. and 10 a.m. 1.25 mCi/kg body weight of the drug was infused through the catheter over 15 s, followed by 0.5 ml 0.9% NaCl solution for 30 s. The rats were anesthetized using 4% halothane in a gaseous mixture of 2 l N_2O and 1 l O_2 /min. In consecutive order liver, tumor, left kidney, spleen, a piece of small intestine, thymus and femoral bone marrow were removed and immersed in liquid nitrogen.

Chemical Analyses. The acid-soluble fraction and RNA were extracted according to Munro and Fleck (1966). The radioactivity was measured in a Packard (Downers Grove, Ill.) Tricarb CD 300 liquid scintillation system. The acid-soluble fraction contains all compounds soluble in 0.1 M cold perchloric acid. This fraction and RNA were calculated as μg of ultraviolet absorbing substances. DNA was

analyzed using the technique of Scott et al. 1956 as modified by Hinrichs et al. 1964.

The dpm was divided by 2.22×10^6 the sp. act. of the purchased substance to give the amount of administered substance in the target molecules of given substance.

Statistical Analyses. The Student's two-tailed t -test was employed on the logarithmic values.

Results

The results are given in Table 1. Fura and Furd gave greater labeling of the acid-soluble fraction of all tissues, especially liver and kidney, than the corresponding natural compounds. Fdurd gave greater labeling of this fraction in liver and kidney than deoxyuridine.

Fura gave a substantially heavier labeling of tumor RNA than uracil. Fdurd gave a lower labeling than deoxyuridine of DNA, but a heavier labeling of RNA in all tissues except liver. Orotic acid gave a heavier labeling than all the other compounds of RNA in tumor, liver, and kidney.

Table 1. Amount of given substance as fmole in the acid-soluble fraction, RNA and DNA/mg DNA, corrected for nmole given dose, 90 min after administration of the precursor. The Table gives the mean $\pm$ standard error of the mean. n = number of rats

Pre-cursor	Variable	Tumor	Liver	Bone marrow	Small intestine	Kidney	Spleen	Thymus	n
Uracil	ASF	325 ± 42	686 ± 35	65.2 ± 3.7	143 ± 5	308 ± 8	116 ± 4	81.6 ± 6.1	6
	RNA	7.68 ± 1.07	23.2 ± 2.8	1.73 ± 0.10	3.47 ± 0.24	3.20 ± 0.31	0.959 ± 0.063	0.458 ± 0.068	
	DNA	0.866 ± 0.157	4.29 ± 0.74	1.16 ± 0.07	1.09 ± 0.06	0.769 ± 0.80	0.746 ± 0.057	0.70 ± 0.013	
FUra	ASF	953 ± 123^a	$10,301 \pm 799^a$	154 ± 6^a	374 ± 48^a	6605 ± 541^a	215 ± 12^a	192 ± 16^a	6
	RNA	225 ± 50^a	49.8 ± 6.4^a	2.98 ± 0.27^a	14.1 ± 10.5	9.25 ± 1.70^b	4.12 ± 2.63	0.104 ± 0.074^c	
	DNA	0.553 ± 0.182	5.98 ± 1.08	0.045 ± 0.024^b	0.771 ± 0.435	0.324 ± 0.140	0.476 ± 0.130	nm	
Uridine	ASF	569 ± 64	1413 ± 187	68.5 ± 4.5	151 ± 6	382 ± 42	112 ± 4	67.9 ± 4.5	6
	RNA	160 ± 21	224 ± 49	2.8 ± 0.10	4.99 ± 0.31	7.62 ± 0.96	5.15 ± 3.28	0.864 ± 0.273	
	DNA	9.72 ± 1.89	10.2 ± 1.5	2.21 ± 0.23	1.44 ± 0.07	1.18 ± 0.09	1.70 ± 0.55	0.114 ± 0.039	
Furd	ASF	2031 ± 288^a	$12,114 \pm 908^a$	139 ± 7^a	352 ± 29^a	6213 ± 560^a	253 ± 16^a	978 ± 364^a	5
	RNA	718 ± 84^a	262 ± 42	8.58 ± 1.41^a	11.2 ± 4.0	38.9 ± 4.8^a	6.36 ± 1.53	3.82 ± 1.59	
	DNA	2.83 ± 0.40^b	8.80 ± 1.60	1.32 ± 0.42	1.14 ± 0.61	0.811 ± 0.280	2.24 ± 0.41	nm	
Deoxy-uridine	ASF	1447 ± 70	4356 ± 103	360 ± 10	790 ± 41	1784 ± 27	532 ± 17	364 ± 17	6
	RNA	54.3 ± 10.4	133 ± 5	10.0 ± 1.1	11.0 ± 0.6	8.37 ± 0.47	4.51 ± 0.84	1.31 ± 0.20	
	DNA	1047 ± 222	47.3 ± 15.1	329 ± 27	52.9 ± 12.5	5.66 ± 1.29	105 ± 44	8.47 ± 3.49	
Fdurd	ASF	1667 ± 523	$19,360 \pm 1402^a$	202 ± 9^a	461 ± 16^a	$10,483 \pm 648^a$	292 ± 17^a	231 ± 22^a	7
	RNA	636 ± 85^a	265 ± 67	485 ± 70^a	140 ± 7^a	65.9 ± 9.1^a	224 ± 49^a	12.6 ± 1.2^a	
	DNA	20.4 ± 2.6^a	8.82 ± 1.96^a	23.2 ± 2.6^a	6.19 ± 0.23^a	1.92 ± 0.36^b	15.4 ± 2.4^b	0.282 ± 0.117^b	
Orotic acid	ASF	4991 ± 1212	$41,989 \pm 4191$	76.7 ± 12.9	171 ± 7	$11,955 \pm 2345$	179 ± 45	104 ± 20	6
	RNA	1163 ± 304	7379 ± 842	15.8 ± 4.7	16.1 ± 2.5	2060 ± 433	13.0 ± 5.3	4.99 ± 1.73	
	DNA	10.4 ± 2.5	173 ± 26	4.27 ± 0.86	0.153 ± 0.054	43.9 ± 10.2	1.63 ± 0.35	nm	

^a $P < 0.001$

^b $0.001 < P < 0.01$

^c $0.01 < P < 0.02$ compared to corresponding natural substance

nm = not measurable

Discussion

The high labeling of the acid-soluble fraction by FURA and FdUrd is interesting, possibly indicating a decreased utilization for or synthesis of nucleic acid. The especially high labeling of the acid-soluble fraction of liver and kidney might also represent excretion.

Interestingly, FdUrd gave greater labeling of RNA than deoxyuridine in all tissues except liver. Probably FdUrd is directed towards RNA synthesis after metabolism, as it cannot be utilized properly for DNA synthesis.

The ultimate goal of treatment with cytostatic agents is to get as much of the drug as possible into the tumor and its target molecules and as little as possible into normal cells. The dose-limiting toxicity of 5-fluoropyrimidines appears to be mainly gastrointestinal and hematopoietic (Kirkwood et al. 1980; Martin et al. 1980; Au et al. 1982). In several tumor systems the cytotoxicity of these compounds seems to be related to their incorporation into RNA (Ardalan et al. 1981; Kufe et al. 1981; Glazer et al. 1982). Another factor is inhibition of DNA synthesis (Heidelberger et al. 1983), although there is some incorporation into DNA (Sawyer et al. 1984).

In the present experiment the substances were given in equal radioactivities, not in equal molarities. The liver contains 240 nmol UTP/mg DNA (Christensson et al. 1985), corresponding to 5,000 nmoles/liver in a 200 g rat. Our rats were given 5 to 17 nmoles of the substances. The highest amount of given substance was obtained in the acid-soluble fraction of liver, namely 0.04 nmoles/mg DNA after orotic acid administration. The amounts given can therefore be regarded as tracer doses. There were no obvious differences between FdUrd and FURA in the ratio of labeling of tumor RNA in relation to normal tissue RNA. FURA was therefore chosen for experiments with therapeutic doses, now in progress.

Various combinations of drugs, for example FURA plus FdUrd, can also be tested, if different tracer atoms (^3H and ^{14}C) are utilized. A potential drug can be infused via the hepatic artery and simultaneously protecting substances as deoxyuridine and uridine via a femoral vein.

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EFFECTS OF DOSAGE AND INFUSION TIME OF 5-FLUOROURACIL ON DNA AND RNA OF NORMAL TISSUES AND AN ADENOCARCINOMA TRANSPLANTED TO RAT LIVER

Christian Erichsen¹, MD, Per-Inge Christensson², MD, Birgitta Jacobsson², MD, Per-Ebbe Jönsson^{2,3}, MD, Unne Stenram², MD.

Departments of Surgery¹ and Pathology², University of Lund, S-221 85 LUND, Sweden and Department of Surgery³, Helsingborg Hospital, 251 87 Helsingborg, Sweden

Offprint request to Christian Erichsen, Department of Surgery, S-221 85, Lund, Sweden

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Abbreviations: FUra, fluorouracil; FdUMP, fluoro-2¹-deoxy-uridine monophosphate

In a model of secondary liver cancer in Wistar rats the incorporation of 5-FUra into the acid soluble fraction, RNA and DNA of several normal tissues and of an adenocarcinoma of the colon transplanted to the liver was determined. 300, 1 200 or 24 000 nmole 5-FUra was infused via the gastroduodenal artery for 0.5, 2 or 24 h. The rats were killed 1.5, 3 or 24 h after the beginning of the infusions. In general, higher doses gave a higher labelling. However, the ratio of incorporation into tumor compared to normal tissue RNA was higher at the 1 200 than at the 24 000 nmole dosage. There was a decreased RNA/DNA ratio in the tumor at 24 h after 24 000 nmole was infused over 0.5 or 2 h, indicating decreased synthesis and/or increased breakdown of RNA.

INTRODUCTION

Liver metastases from colorectal cancer are a major clinical problem as about 20 - 25 % of the patients have metastases at the time of primary surgery and another 20 - 25 % will develop metastases later on (Bengmark et al 1969, Bengtsson et al 1981). The role of 5-fluoropyrimidines, especially 5-FUra alone or combined with other cytostatic agents in managing liver metastases from colorectal cancer, has been established (Gustavsson et al 1981, Patt et al 1983). Numerous studies of regional infusion into the hepatic artery of 5-FUra have demonstrated that high drug concentrations can be delivered to the liver by this approach with objective tumor response without increasing systemic toxicity (Ensminger et al 1978, Balch et al 1983). This can be explained by the preferentially arterial blood supply of the metastases and by fluoropyrimidines being metabolized and cleared by the liver. Even if the use of regional infusional

therapy of liver metastases is increasingly spread around the world, the question about the therapeutic benefits is still unanswered. In a recent study multivariate analyses have been used to define patients suitable for such treatment. Prolonged intraarterial infusion of 5-FUra in patients with colorectal liver metastases was demonstrated to improve the survival time significantly compared to intermittent high-dose treatment by the same route (Ekberg et al 1986). After long-term infusion the risk of toxic hepatitis and sclerosing cholangitis is increased (Kemeny et al 1985).

Pharmacokinetic studies with concomitant analyses of the drug level in hepatic and peripheral veins have been performed both in animals and humans and have confirmed that the hepatic artery is the optimal route of drug administration (Ensminger et al 1978, Gustavsson et al 1979, Didolkar et al 1984). Differences may exist between humans and animals, but the clearance of 5-FUra seems to be similar. Regarding dose schedules and the length of infusion time many questions can be raised. The antitumor effect depends on the incorporation of the drug into RNA and the inhibition of DNA synthesis. The optimal effects on tumor in relation to normal cells cannot be evaluated by analyses of drug levels in blood. The relationship between the dose and the length of infusion time for maximum tumor effect and minimum toxicity has to be found. With the aid of an experimental model of a colon adenocarcinoma transplanted to the liver, which mimics the clinical situation, it is possible to settle this more exactly. The aim of this experimental study was to explore the influence of different doses and lengths of infusion time on the incorporation of 5-FUra into RNA and its effect on DNA of tumor and normal tissues.

Animals and tumor

Inbred female rats of Wistar strain weighing 175-225 g were used for the experiments. The rats were kept individually and were given access to standard laboratory diet and tap water ad libitum. The tumor used in the study was an N-methyl-nitrosoguanidine-induced adenocarcinoma of the colon (Steele et al 1974). In all rats a suspension containing 1.0×10^6 viable tumor cells was inoculated in the periphery of the central lobe of the liver just under the capsule (Carlsson et al 1983) under ether anesthesia.

Chemicals

5-6- ^3H -FUra (16.5 Ci/mmol) was purchased from New England Nuclear Corp, Boston, Mass. 5-FUra and ^3H -FUra were kindly supplied by Hoffman-La Roche, Switzerland. All chemicals were pro analysi.

Experimental design

At laparotomy nine days after the tumor inoculation, a catheter (Intramedic, PE 10; Clay Adams; Parsippany, NJ) was placed into the gastroduodenal artery under halothane anesthesia (Christensson et al 1985). At this time the tumor volume was about 0.25 cm^3 when calculated according to the formula $V = a \times b^2/2$, where a is the largest and b the smallest diameter (Carlsson et al 1983). The animals were placed in individual cages with a catheter connected to an infusion pump (Braun AG, type 1850 FRG) giving 1 ml 0.9 % NaCl solution/24 h. Heparin 10 IU/ml was added to prevent catheter occlusion and artery thrombosis. Three days later the rats

were randomized to three different dose schedules of 5-FUra; 300, 1200 and 24000 nmol. This was administered through the catheter by an STC 521 pump (Terumo, Japan), with different lengths of infusion time; 0.5, 2 and 24 h. 84 rats were used in all. Most rats were sacrificed 24 h after start of the infusion, some of them 1 h after the end of the short infusions (Tables). The rats were anesthetized using 4% halothane in gaseous mixture of 2 l N₂O and 1 l O₂/min.

A piece of the liver was frozen in liquid nitrogen. Then the tumor was cut clean from the liver within 45-60 seconds and in a few seconds cut into small pieces with a pair of scissors and frozen in liquid nitrogen. In consecutive order the right kidney, a piece of small intestine and femoral bone marrow were removed and immersed in liquid nitrogen. The frozen liver was thawed to about 0°C, pressed through a plastic tube with 1 mm bottom holes to remove connective tissue, mixed by stirring and refrozen (Willén 1970). The procedure was performed in a cold room.

Chemical analyses

The acid soluble fraction and RNA were extracted according to Schmidt and Thannhauser as modified by Munro and Fleck 1966. Munro and Fleck found that an extinction of 1.000 at 260 nm corresponds to 32 ug RNA/ml. We have found that this value is also valid for UV-absorbing substances in the acid soluble fraction. In the Tables ug ASF refers to ultraviolet-absorbing substances in this fraction. DNA was analyzed according to Scott et al 1956 as modified by Hinrichs et al 1964.

The radioactivity was measured in a Packard (Downers Grove, Ill.) TriCarb CD 300 liquid scintillation system. The dpm was divided by 2.22 x the

specific activity to give the amount of administered substance from the labelled batch. This had to be multiplied by the amount of cold FUra to give the amount of a given substance incorporated into the fractions.

Statistical analyses

Student's two-tailed t-test was employed.

RESULTS

The results are given in the Tables. In general there is an increased incorporation of 5-FUra into the acid soluble, RNA and DNA fractions with increasing doses, though with some differences among the tissues (Table I). Thus the ratio of incorporation into tumor RNA compared to normal tissue RNA is always higher with a 1200 nmole than with a 24 000 nmole dose (Table 2) .

The ratios of RNA and of the ultraviolet-absorbing substances of the acid soluble fraction to DNA are also given in Table I. There was only one difference between the groups. At the 24 000 nmole dosage given for 0.5 and 2 h and with a labelling time of 24 h the RNA/DNA ratio in tumor decreased in both groups to 0.74 ± 0.10 and 0.74 ± 0.08 , respectively. The value found in these two groups combined (0.74 ± 0.06) is significantly different ($P < 0.001$) from that in the other groups combined (1.05 ± 0.03).

DISCUSSION

The ultimate goal of treatment with cytostatic agents is to get as much of the drug as possible into the tumor and its target molecules and as little as possible into normal cells. The dose limiting toxicity of 5-fluoropyrimidines appears to be mainly gastrointestinal and hematopoietic (Kirkwood et al 1980, Martin et al 1980, Au et al 1982). In several tumor systems the cytotoxicity of these compounds seems to be related to their incorporation into RNA (Ardalan et al 1981, Kufe et al 1981, Glazer et al 1982). Another factor is inhibition of DNA synthesis (Heidelberger et al 1983), although there is some incorporation into DNA (Schuetz et al 1985). It was recently claimed that after exposure for one hour to 5-FUra, cytotoxicity correlates more closely to incorporation into the acid soluble fraction than to incorporation into RNA (Meisler et al 1985). The incorporation of 5-FUra into RNA and perhaps also the acid soluble fraction may therefore be a measure of the cytotoxicity. DNA synthesis is inhibited by formation of a thymidylate synthetase: FdUMP : N⁵N¹⁰-methylene tetrahydrofolate complex, which is considered to be found in the DNA fraction with the method employed (Parker et al 1985). This interpretation agrees with our previous studies with 5-fluoropyrimidines (Erichsen et al 1986). As some incorporation of FUra into DNA is also said to take place, the labelling of the DNA fraction may also be taken as a measure of cytotoxicity.

The incorporation of FUra into RNA did not increase linearly with the dosage. It is, however, not only a question of uptake into the cells. 5-fluoropyrimidines decrease the RNA synthesis (Iapalucci-Espinoza et al 1982, Eriksson & Stenram 1984), and thus counteract the incorporation into RNA. A premature breakdown of false RNA is also produced. 5-FUra increases nucleolar size, delays the processing of nucleolar RNA and

prevents the formation of ribosomes and ribosomal RNA (Stenram 1966, Stenram et al 1970, 1972, Wilkinson et al 1976). The time at which the labelling is measured is thus of importance and it may be of interest to examine if the cytoplasmic ribosomal RNA is labelled. Interestingly there was a decreased RNA content of the tumor cells at the 24 000 nmole dosage and short infusion time, i.e. when there was a considerable time for effects on the body.

The acid soluble fraction is the one showing the highest incorporation of FUra, especially in the liver and the kidney. The high labelling of the acid-soluble fraction in liver and kidney might to some extent represent excretion. The ratio of incorporation into RNA to incorporation into the acid soluble fraction is in general lower for liver than for tumor. The ratio of incorporation into RNA of tumor compared to normal tissue was always higher for the 1200 than for the 24000 nmol dosage, suggesting an improved therapeutic index with the former dosage. The 24 000 nmole dosage corresponds to the systemic dose given to humans. 1 200 nmole corresponds to this dose when given to the liver alone. The infusion time of 2 h with a dose of 1200 nmol seems to be suitable for further studies on the present topic.

With the present tumor model various combinations of drugs, e.g. FUra + FdURD can be tested if different tracer atoms (^3H and ^{14}C) are utilized. A potential drug can be infused via the hepatic artery and protecting substances as deoxyuridine and uridine by the intravenous route simultaneously. Another important factor, especially in the labelling of normal tissues, is the state of nutrition and future studies should also be aimed at this question.

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Table 1. Amount of given Flura as picomole in the acid-soluble fraction, RNA and DNA/mg DNA. Labelling time is from start of infusion till sacrifice. The Table gives the mean $\pm$ standard error of the mean. The ratio of ultraviolet-absorbing substances (NU) in the acid-soluble fraction and RNA to DNA is also given. n = number of rats.

Infusion time and infusion time	Labelling time	Variable	Tumor	Liver	Bone marrow	Small intestine	Kidney	(n)
100 nmole 30 min	90 min	ASF RNA DNA	436 $\pm$ 49 55 $\pm$ 5 0.61 $\pm$ 0.08	2736 $\pm$ 251 28 $\pm$ 7 3.3 $\pm$ 1.6	109 $\pm$ 17 7.7 $\pm$ 2.3 1.3 $\pm$ 0.1	282 $\pm$ 76 13.1 $\pm$ 5.3 1.5 $\pm$ 0.7	1262 $\pm$ 247 7.8 $\pm$ 1.4 0.64 $\pm$ 0.14	7
100 nmole 30 min	90 min	ASF RNA DNA	1489 $\pm$ 370 274 $\pm$ 58 2.2 $\pm$ 0.5	10982 $\pm$ 3216 46 $\pm$ 13 3.6 $\pm$ 0.7	155 $\pm$ 30 11.5 $\pm$ 7.3 3.7 $\pm$ 2.3	362 $\pm$ 34 5.1 $\pm$ 1.3 0.84 $\pm$ 0.14	7727 $\pm$ 2566 9.2 $\pm$ 1.3 0.53 $\pm$ 0.05	7
200 nmole 30 min	90 min	ASF RNA DNA	15637 $\pm$ 2851 2677 $\pm$ 713 14.4 $\pm$ 3.7	223857 $\pm$ 13326 848 $\pm$ 125 84 $\pm$ 16	3022 $\pm$ 70 205 $\pm$ 9 36 $\pm$ 1	5895 $\pm$ 231 124 $\pm$ 12 8.4 $\pm$ 0.7	221907 $\pm$ 27497 298 $\pm$ 24 19.0 $\pm$ 2.8	4
300 nmole 30 min	24 h	ASF RNA DNA	104 $\pm$ 5 30 $\pm$ 13 1.0 $\pm$ 0.5	303 $\pm$ 29 9.2 $\pm$ 4.5 1.4 $\pm$ 0.9	30 $\pm$ 2 3.6 $\pm$ 1.9 2.0 $\pm$ 1.0	49 $\pm$ 6 1.2 $\pm$ 0.2 0.27 $\pm$ 0.04	134 $\pm$ 11 4.6 $\pm$ 2.5 0.45 $\pm$ 0.21	6
1000 nmole 30 min	24 h	ASF RNA DNA	450 $\pm$ 34 86 $\pm$ 32 1.5 $\pm$ 0.6	1488 $\pm$ 70 27 $\pm$ 5 4.7 $\pm$ 2.4	138 $\pm$ 6 7.5 $\pm$ 1.4 2.3 $\pm$ 1.0	228 $\pm$ 11 9.9 $\pm$ 3.8 0.78 $\pm$ 0.38	594 $\pm$ 48 5.9 $\pm$ 1.5 0.65 $\pm$ 0.35	7
2000 nmole 30 min	24 h	ASF RNA DNA	11281 $\pm$ 953 210 $\pm$ 836 24 $\pm$ 7	29520 $\pm$ 1802 979 $\pm$ 128 41 $\pm$ 5	2935 $\pm$ 270 271 $\pm$ 29 37 $\pm$ 5	6164 $\pm$ 689 221 $\pm$ 21 17.5 $\pm$ 1.6	14277 $\pm$ 747 247 $\pm$ 15 10.7 $\pm$ 0.7	6

100 nmole	3 h	ASF RNA DNA	1179±118 333±84 3.0±0.6	7742±1072 40±7 6.7±1.9	116±10 5.3±0.2 3.0±0.2	449±128 10.2±3.4 1.8±0.7	5400±780 9.0±0.6 0.76±0.04	9
100 nmole	24 h	ASF RNA DNA	159±16 32±9 1.3±0.8	388±53 18.0±7.4 1.2±0.7	45±7 1.1±0.2 0.34±0.15	71±7 2.5±0.5 0.39±0.09	198±32 0.92±0.43 0.21±0.10	7
100 nmole	24 h	ASF RNA DNA	517±55 251±97 1.9±0.6	1611±142 20±4 0.81±0.62	163±8.3 6.4±1.9 0.91±0.33	279±30 40±4 0.39±0.09	718±72 4.6±1.0 0.18±0.11	7
100 nmole	24 h	ASF RNA DNA	8000±980 1446±517 78±56	23849±2010 953±244 82±38	2231±243 215±39 133±71	4294±532 181±47 49±25	10204±979 242±52 27±12	6
100 nmole	24 h	ASF RNA DNA	136±24 10.8±4.2 nm	803±166 4.5±2.5 nm	37±4 3.6±0.8 1.5±0.3	129±31 2.1±1.2 nm	362±43 1.1±0.6 nm	6
100 nmole	24 h	ASF RNA DNA	522±97 91±20 0.96±0.19	2980±308 23±2 1.9±0.4	148±23 5.5±1.1 2.5±0.4	286±49 6.2±1.6 0.80±0.21	1647±279 6.0±0.6 0.66±0.24	6
100 nmole	24 h	ASF RNA DNA	7978±1037 689±109 17.1±2.6	47448±5437 527±86 60±14	2728±263 159±22 52±6	5527±579 157±42 19±5	26928±4137 198±22 27±27	6
100 nmole	24 h	mgNT/mgDNA mgRNA/mgDNA	0.30±0.01 1.00±0.03	1.44±0.02 4.12±0.05	0.19±0.00 0.58±0.01	0.33±0.01 0.79±0.01	0.68±0.01 0.99±0.01	84 84

measurable

Table 2. Tumor: normal tissue ratios with respect to labelling in the acid-soluble fraction and in RNA. The Table gives the mean $\pm$ standard error of the mean. n = number of rats.

Dose and infusion time	Labelling time	Variable	Tumor/liver	Tumor/Bone marrow	Tumor/Small intestine	(n)
300 nmole 30 min	90 min	ASF RNA	0.18 $\pm$ 0.04 2.9 $\pm$ 0.8	4.3 $\pm$ 0.4 18 $\pm$ 7	2.0 $\pm$ 0.3 22 $\pm$ 10	7
1200 nmole 30 min	90 min	ASF RNA	0.21 $\pm$ 0.04 8.1 $\pm$ 2.0	7.7 $\pm$ 1.5 65 $\pm$ 13	4.0 $\pm$ 0.8 79 $\pm$ 27	7
24000 nmole 30 min	90 min	ASF RNA	0.07 $\pm$ 0.01 3.6 $\pm$ 1.1	5.2 $\pm$ 1.0 13 $\pm$ 4	2.9 $\pm$ 0.4 22 $\pm$ 6	4
300 nmole 30 min	24 h	ASF RNA	0.36 $\pm$ 0.04 5.8 $\pm$ 2.7	3.6 $\pm$ 0.3 85 $\pm$ 66	2.3 $\pm$ 0.3 31 $\pm$ 15	6
1200 nmole 30 min	24 h	ASF RNA	0.30 $\pm$ 0.02 3.3 $\pm$ 1.1	3.3 $\pm$ 0.3 17 $\pm$ 8	2.0 $\pm$ 0.2 20 $\pm$ 9	7
24000 nmole 30 min	24 h	ASF RNA	0.39 $\pm$ 0.05 2.4 $\pm$ 0.9	3.9 $\pm$ 0.2 7.8 $\pm$ 3.3	1.9 $\pm$ 0.2 9.2 $\pm$ 3.5	6

1200 nmole 2 h	3 h	ASF RNA	0.20±0.04 9.8±2.0	11.3±1.6 72±19	3.7±0.7 50±11	9
300 nmole 2 h	24 h	ASF RNA	0.41±0.03 3.1±1.3	3.6±0.6 51±26	2.1±0.2 18±7	7
1200 nmole 2 h	24 h	ASF RNA	0.32±0.01 1.3±4	3.2±0.3 58±20	1.9±0.2 66±31	7
24000 nmole 2 h	24 h	ASF RNA	0.33±0.03 2.1±0.8	3.6±0.2 7.6±2.8	2.0±0.3 11±5	6
300 nmole 24 h	24 h	ASF RNA	0.19±0.05 3.8±2.3	3.9±0.3 4.4±1.9	1.3±0.5 5.2±2.3	6
1200 nmole 24 h	24 h	ASF RNA	0.17±0.02 4.2±0.9	3.5±0.3 20±5	1.8±0.2 18±5	6
24000 nmole 24 h	24 h	ASF RNA	0.17±0.01 1.4±0.2	3.0±0.3 5.0±1.0	1.5±0.2 5.6±1.5	6

Effect of PALA and D-Glucosamine on Uracil Nucleotides and the
Incorporation of 5-Fluorouridine into Normal Tissues and an
Adenocarcinoma transplanted into the Liver in Rat.

Christian Erichsen¹, Per Inge Christensson²,
Birgitta Jakobsson², Göran Eriksson², Torsten Yngner¹,
Per Ebbe Jönsson³, Unne Stenram².

Departments of Surgery¹ and Pathology² in Lund
University of Lund,
S-221 85 LUND
SWEDEN

Department of Surgery³
Helsingborg Hospital
S-251 87 HELSINGBORG
SWEDEN

Offprint requests to: Christian Erichsen, Department of Surgery,
University of Lund, S-221 85 LUND, Sweden.

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Abbreviations: PALA, N-phosphonacetyl-L-aspartate; FUrd,
fluorouridine; FdUMP, fluoro-2'-deoxyuridine monophosphate.

Summary. Cytostatic treatment of liver metastases from colorectal cancer has hitherto been of limited value. Higher drug levels in the target substances of the tumor may improve the results. It was the aim of this investigation to examine the effect of PALA (N-phosphonacetyl-L-aspartate) and D-glucosamine 1) on the level of UTP in liver and in an N-methyl-N-nitrosoguanidine-induced adenocarcinoma of the colon transplanted to the liver of rat and 2) on the incorporation of ^3H -FUrD into the acid-soluble, the RNA and the DNA fractions of the tumor and several normal tissues. Drugs were administered via the gastroduodenal artery.

Uracil nucleotides in liver and tumor were measured with isotachophoresis.

Combined treatment with PALA and D-glucosamine reduced the UTP pool and increased UDP-N-acetylhexosamine in liver tissue. Pre-treatment with PALA and D-glucosamine increased incorporation of ^3H -FUrD into RNA of the liver and kidney, but not of the tumor, and the DNA fraction of the liver.

Key words: 5-Fluoropyrimidines - PALA (N-phosphonacetyl-L-aspartate) - D-glucosamine - Nucleotides - RNA - DNA - Liver metastases

INTRODUCTION

Up to one fourth of patients with colorectal cancer have liver metastases at the time of diagnosis and an equal number will subsequently develop them (Bengtsson et al. 1981). Despite the use of improved surgical techniques together with different cytostatic agents, therapeutic failures in cases with metastases remain disappointingly high.

The most consistently used drugs for such treatment are 5-fluoropyrimidines (Gustavsson et al. 1981). Their cytotoxicity is related to incorporation into RNA and interaction with DNA synthesis by inhibition of thymidylate synthetase (Heidelberger et al. 1983) as well as possibly to incorporation into DNA (Sawyer et al. 1984) and incorporation into the acid-soluble fraction (Meisler et al. 1985). Since more efficient drugs have not been developed, it is mandatory to find ways to increase the effect of the available ones.

An ideal cytostatic agent should selectively be taken up by tumor cells and only minimally by normal tissue cells. In addition, increased incorporation of the cytostatic agent into the target substances in the tumor cells might possibly be achieved by interfering with certain metabolic pathways. The UTP pool could be depleted by pretreatment with PALA, by interference with UTP synthesis, by blockade of the enzyme aspartate transcarbamylase and by D-glucosamine, which consumes UTP by forming UDP-N-acetylglucosamine (Collins et al. 1971; Keppler et al. 1985). A depleted UTP pool, decreasing the competitive inhibition of uridine kinase, would enhance the effect of the enzymes which regulate the uptake of uridine and 5-FUrd into the tumor cells and their phosphorylation (Anukarahanonta et al. 1980). PALA might also influence DNA synthesis by the reduction of the nucleotide pool (Moyer et al. 1982). D-glucosamine might also exert separate effects on cellular membrane synthesis by interference with glycoprotein and glycolipid synthesis (Friedman et al. 1980). In a previous study with an adenocarcinoma in the rat liver, pretreatment with PALA and D-glucosamine potentiated the inhibitory effect of 5-FUrd on tumor growth

(Erichsen et al. 1982).

Clinically, PALA has been used either alone with moderate toxicity (Loo et al. 1980; Ehrlichman et al. 1979) or in combination with 5-FUra (Bedikian et al. 1981; Caspar et al. 1983). However, only limited therapeutic effects were registered in patients with colorectal cancer (Chiuten 1985; Schein 1985; Shah et al. 1985). The explanation for this could be incorrect treatment schedules and/or drug combinations. Therefore it is of utmost importance to further investigate these drugs in experimental animals.

The present study concerns the incorporation of a tracer dose of ^3H -FUrd into the acid-soluble, the RNA and the DNA fractions of several normal tissues and of an adenocarcinoma of rat colon inoculated into the liver, after pretreatment with PALA and D-glucosamine. In preliminary experiments the effects of PALA and/or D-glucosamine on the UTP level were determined in liver and tumor.

MATERIAL AND METHODS

Animals and Tumors

Inbred female rats of the Wistar strain weighing 145-210 g were used for the experiments. The rats were kept individually and were given access to standard laboratory diet and tap water ad libitum. An N-methyl-N-nitrosoguanidine induced transplantable adenocarcinoma of rat colon was used (Steele et al. 1974). Under ether anesthesia a cell suspension containing 1.0×10^6 tumor cells in 0.1 ml 0.9% NaCl was injected into the central liver lobe through a midline incision.

Chemicals

5-6- ^3H -FUrd (17.4 Ci/mmol) was purchased from New England Nuclear Corporation (Boston, Mass.). The substance was delivered in 70% ethanol. It was freeze-dried overnight and dissolved in sterile water immediately before use. D-glucosamine and PALA were obtained

from Calbiochem (La Jolla, Cal.). All chemicals used were pro analysi.

Experimental Design

At laparotomy 10 days after tumor inoculation, a catheter (Intra-medec PE 10; Clay Adams, Parsippany, N.J.) was placed into the gastroduodenal artery. At this time the tumor volume was about 0.25 cm^3 , when calculated according to the formula $V = A \times B^2 / 2$, where A is the largest and B the smallest diameter (Carlsson et al. 1983). The animals were placed in individual cages with the catheter connected to an infusion pump (type 1850, Braun AG, FRG), giving 1 ml 0.9% NaCl solution per 24 h. All subsequent infusions were given through the catheter. The infusion time for each drug or tracer was 15 s, followed by 0.5 ml 0.9% NaCl solution for another 15 s. Two experiments were performed.

After several preliminary trials with different dosages and time intervals, the first experiment was performed with 3 rats given PALA, 200 $\mu\text{mol/kg}$ body weight, and 3 rats D-glucosamine, 10 mmol/kg body weight, 4 rats both substances and 4 rats 0.9% NaCl. The rats were sacrificed one h after administration of D-glucosamine and 24 h after administration of PALA, i.e. the time interval between PALA and D-glucosamine was 23 h. They had been anesthetized in a halothane- $\text{N}_2\text{O}-\text{O}_2$ atmosphere (Christensson et al. 1985). A piece of the liver was taken out within 5 s and freeze-clamped. The tumor was then cut clean from the liver within 45-60 s and freeze-clamped.

In the second experiment 5 rats were allocated to pretreatment with PALA and D-glucosamine. The infusion of PALA (200 μmol per kg body weight) was started eight h after the laparotomy for catheter implantation. 23 h after PALA administration, these rats received D-glucosamine (10 mmol per kg body weight) and after another hour they were given a tracer dose of ^3H -Furd (0.15 mCi). Four control animals received 0.9% NaCl instead of PALA and D-glucosamine. After the labelling period of 90 min, a piece of the liver was

frozen in liquid nitrogen. The tumor was taken out in a few seconds, divided into small pieces with a pair of scissors and frozen in liquid nitrogen. In consecutive order, the right kidney, a piece of cleaned small intestine and femoral bone marrow were removed and immersed in liquid nitrogen.

Chemical Analyses

In experiment 1 nucleotides were extracted and analyzed by isotachophoresis according to Eriksson (1980, 1984) but with a column coupling system using a quartz capillary.

The acid-soluble fraction and RNA were extracted according to Munro and Fleck (1966). This fraction and RNA were calculated as μg of ultraviolet absorbing substances. Munro and Fleck found that an extinction of 1.000 at 260 nm corresponds to 32 μg RNA/ml. We have found that this value is also valid for UV-absorbing substances in the acid-soluble fraction.

DNA was analyzed by the technique of Scott et al. (1956) as modified by Hinrichs et al. (1964).

The radioactivity was measured in a Packard (Downers Grove, Ill.) TriCarb CD 300 liquid scintillation system.

Statistical Analyses

Student's two-tailed t-test was employed on the logarithmic values.

RESULTS

The isotachophoretic analyses showed a value of 72 nmol/mg DNA of UTP in the liver of control rats. This value decreased by 35% with PALA treatment and by 45% with combined treatment with PALA and D-glucosamine.

UDP-N-acetylhexosamine was 600 nmol/mg DNA in the control rats and increased 50% following combined treatment with PALA and D-glucosamine and almost three-fold with D-glucosamine only ($0.01 > p > 0.001$).

Low values were obtained in tumor tissue. However, UDP-N-acetylhexosamine increased from 115 nmol/mg DNA in control rats to 290 after D-glucosamine treatment.

The effect of PALA and D-glucosamine on the incorporation of ^3H -FURd into the acid-soluble, RNA and DNA fractions of tumor and other tissues after intraarterial administration is shown in table 1. There was no increase in the labelling of the acid-soluble fraction, of RNA or of DNA in the tumor, bone marrow or small intestine. In liver, PALA plus D-glucosamine increased the labelling of RNA fivefold. In the kidney the labelling of the acid-soluble fraction was significantly reduced and that of RNA significantly increased after PALA and D-glucosamine pretreatment. The labelling of the DNA fraction was significantly increased in the liver.

DISCUSSION

Isotachophoretic analysis clearly demonstrated that combined treatment with PALA and D-glucosamine decreased the UTP and increased the UDP-N-acetylhexosamine pool in the liver of the tumor-bearing rats, in agreement with previous studies (Bekesi et al. 1969, Keppler et al. 1970). These dosages and time intervals were thus thought suitable for studies of possible effects on 5-FURd metabolism. The nucleotide values obtained were, however, slightly

different from those obtained in non-tumor-bearing rats by freeze-clamping in situ (Christensson and Stenram 1985) - a procedure not possible to perform here with the equipment available due to the tumor.

In agreement with the UTP-depressing effects of PALA and D-glucosamine, the RNA labelling of the liver increased after pretreatment with these two substances. The labelling of the DNA fraction also increased. It has been claimed that the thymidylate synthetase: $\text{FdUMP:N}^5, \text{N}^{10}$ -methylenetetrahydrofolate complex is found in this fraction (Parker et al. 1985). It is therefore very interesting that the labelling of the DNA fraction increased simultaneously with the RNA labelling, indicating an increased availability of FUr d metabolites for both RNA and any attempted DNA synthesis under the influence of PALA and D-glucosamine.

In the kidney there was also an increased RNA labelling and a tendency to increased labelling of the DNA fraction after PALA and D-glucosamine. The decrease in the labelling of the acid-soluble fraction of the kidney might possibly be due to greater uptake of ^3H -FUr d by the liver. Loo et al (1980) have found high excretion of PALA in the urine, which might imply uptake of PALA into the kidney cells, resulting in increased utilization of ^3H -FUr d for RNA synthesis.

PALA and D-glucosamine had no effect on the labelling of the tumor. Pyrimidines are to a large extent metabolized in the liver (Cooper 1972; Keppler et al. 1982). The experiments with PALA and uridylate-trapping sugar analogs referred to in the introduction have mainly been performed with liver and hepatoma cells. Perhaps it is not surprising therefore that no effects were obtained on the measured parameters (Table 1) in the present tumor, but instead signs of increased toxicity on liver and kidney. This underlines the desirability of knowing the biochemistry of human tumors. It would be of interest to examine nucleotides and their enzymes in experimental and human tumors which are considered suitable for treatment with 5-fluoropyrimidines.

It therefore seems to us that the increased inhibitory effect of 5-FUrd on the growth in this tumor in rats pretreated with PALA and D-glucosamine (Erichsen et al. 1982) cannot be related to enhanced incorporation of 5-FUrd into tumor cell RNA. PALA and D-glucosamine might contribute to the cytotoxic effect of 5-FUrd by other mechanisms cited in the introduction as, for instance, interference with RNA, DNA and cell membrane synthesis (Friedman 1980; Moyer et al. 1982; Chelibonova-Lorer et al. 1983). Further studies of this interrelationship are necessary.

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Table 1. Experiment 2. Effect of treatment with PALA and D-glucosamine (D-Glc) on the incorporation of ^3H -Furd into the acid-soluble fraction (ASF), the RNA and the DNA fraction. The table gives the mean $\pm$ standard error of the mean. n = number of rats.

Variable	Treatment	Tumor	Liver	Bone marrow	Small intestine	Kidney	n
$\frac{\text{dpm in ASF}}{\mu\text{g DNA}}$	PALA + D-Glc control	226 $\pm$ 80 303 $\pm$ 79	3493 $\pm$ 214 3034 $\pm$ 272	47 $\pm$ 5 42 $\pm$ 4	113 $\pm$ 12 126 $\pm$ 6	1122 $\pm$ 53 ^a 2107 $\pm$ 56	5 4
$\frac{\text{dpm in RNA}}{\mu\text{g DNA}}$	PALA + D-Glc control	56 $\pm$ 27 59 $\pm$ 38	119 $\pm$ 21 ^a 23 $\pm$ 2.5	14 $\pm$ 3 10 $\pm$ 4	20 $\pm$ 1.7 23 $\pm$ 1.8	101 $\pm$ 13 ^b 24 $\pm$ 5	5 4
dpm in $\mu\text{g DNA}$	PALA + D-Glc control	0.19 $\pm$ 0.08 0.32 $\pm$ 0.14	2.50 $\pm$ 0.39 ^a 0.82 $\pm$ 0.01	0.48 $\pm$ 0.05 0.57 $\pm$ 0.03	0.12 $\pm$ 0.03 0.20 $\pm$ 0.04	1.25 $\pm$ 0.31 0.41 $\pm$ 0.07	5 4

^a $P < 0.001$

^b $0.001 < P < 0.01$ on the logarithmic values compared to controls

EFFECT OF HEPATIC ARTERY LIGATION ON THE INCORPORATION OF ^3H -OROTIC ACID INTO AN ADENOCARCINOMA TRANSPLANTED TO RAT LIVER

Christian Erichsen, MD¹, Per-Inge Christensson, MD², Per-Ebbe Jönsson, MD^{2,3}, Unne Stenram, MD².

Departments of Surgery¹ and Pathology², University of Lund, S-221 85 Lund, Sweden and Department of Surgery³, Helsingborg Hospital, S-251 87 Helsingborg, Sweden

Correspondence to: Per-Ebbe Jönsson, MD, Department of Surgery, Helsingborg Hospital, S-251 87 Helsingborg, Sweden.

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Key words: ^3H -orotic acid, secondary liver tumor, hepatic artery ligation, portal vein infusion.

ABSTRACT

In the model of secondary liver cancer in Wistar rats a study was made of the influence of hepatic artery ligation (HAL) on the amount of nucleotides and RNA in tumor and liver tissue and on the uptake of ^3H -orotic acid into these compounds and DNA after labelling for 90 minutes. Ten days after inoculation with tumor cells into the central liver lobe, a catheter was placed into the portal vein in all rats and in half of them the hepatic artery was ligated. On days one, three, five or ten, rats were given ^3H -orotic acid through the catheter. On day ten ^3H -orotic acid was also infused via the femoral vein or intraperitoneally. After HAL there was a decrease in the nucleotide and RNA content of the tumor cells after one, three and five days. There was no such decrease in the liver cells. In all HAL rats there was an increase in the nucleotide and RNA content of the tumor cells at day ten compared to day five. The ratio of RNA to acid soluble fraction labelling in tumors was also increased on day ten in all groups compared to HAL rats at day five. The increased uptake of ^3H -orotic acid into tumor RNA at day ten after HAL strongly suggests rearterialization. There was no support for an increased vascularization of the tumor from the portal vein on day three or five. In the liver tissue, HAL had no influence. This experimental study gives no support for the use of hepatic dearterialization followed by intraportal infusion of cytostatic agents in clinical settings.

INTRODUCTION

The blood supply of tumors in the liver, experimental as well as human primary and secondary cancers, has been extensively investigated. There is a general agreement in the literature that these tumors are mainly nourished by the hepatic artery but some disagreement still persists concerning the importance of the portal blood supply (1, 2, 3, 4, 5). However, in the tumor periphery, the possibility of supply from both the arterial and portal vessels has to be considered (4, 5). In treatment of liver metastases from colorectal cancer, hepatic artery ligation (HAL) has been used for several years, either as a single procedure or in combination with intraarterial infusion of cytostatic agents. The clinical value is, however, limited and temporary (6, 7). The shortlived effect when HAL is used alone can be explained by the immediate development of new arterial collaterals in both tumor and normal liver after a few days (8,9,10). In clinical studies HAL in combination with infusion of cytostatic agents via the portal vein has a trend to better survival (11, 12).

In experimental tumor systems regression of tumor and significant prolongation of survival are reported after HAL alone probably due to central tumor necrosis (13). A compensatory increase in portal blood flow to the periphery of the tumor might result in a higher drug exposure to surviving tumor cells following infusion of cytostatic agents through the portal vein. In vitro experiments on isolated nuclei from liver cells recovering from reversible - non-necrogenic -ische-

mia show that RNA synthesis recovers with an overshooting well above normal and lasting at least for 24 hours (14). This situation could be expected in the tumor periphery, and tumor cells in this area may be more susceptible during the recovery phase to drugs like the 5-fluoropyrimidines, which are incorporated into RNA.

This study with an adenocarcinoma of the colon transplanted to the liver in rats was designed to evaluate the effect of HAL on levels of nucleotides and RNA in tumor and liver and on the incorporation of ^3H -orotic acid administered by the portal vein into these compounds. Orotic acid is an important building block in pyrimidine synthesis. It is widely used as an RNA precursor and is also suitable for the tumor employed (15). Its uptake and incorporation into RNA and DNA might indicate how 5-fluoropyrimidines might be taken up.

MATERIAL AND METHODS

Animals and tumor

Inbred female rats of the Wistar strain weighing 170 - 210 g were used for the experiment. The rats were kept individually and were given access to standard laboratory diet and tap water ad libitum. The tumor used in the study was a N-methyl-nitrosoguanidin-induced adenocarcinoma of the colon (16). In all rats a suspension containing 1.0×10^6 viable tumor cells was inoculated in the periphery of the central lobe of the liver just under the capsule (17).

Chemicals

^3H -orotic acid (20 Ci/mmol) was purchased from New England Nuclear Corp., Boston, Mass. All chemicals were pro analysi.

Experimental design

At laparotomy 10 days after the tumor inoculation, a catheter (Intramedic PE 10; Clay Adams, Parsippany, N.J.) was placed into the portal vein under halothane anesthesia (18). In half of the rats the hepatic artery was ligated. At this time the tumor volume was about 0.25 cm^3 when calculated according to the formula $V = a \cdot b^2/2$, where a is the largest and b the smallest diameter (17). The animals were placed in individual cages with a catheter connected to an infusion pump (Braun 1850 FRG) giving 1 ml 0.9 % NaCl solution/24 h. Heparin 10 IU/ml was added to prevent portal vein thromboses. One, three, five or ten days later 0.25 mCi ^3H -orotic acid was given through the catheter. On day 10, six rats were given the tracer by the femoral vein and five by the intraperitoneal route. In total 53 rats were used for chemical analysis (Tables I and II). The infusion time for ^3H -orotic acid was 15 sec, followed by 0.5 ml 0.9 % NaCl solution for another 30 sec. After 90 min the rats were sacrificed in halothane anesthesia (18). A piece of the liver was immediately frozen in liquid nitrogen.

The tumor was cut clean from the liver during 45-60 seconds and in a few seconds cut and divided into small pieces with a pair of scissors and frozen in liquid nitrogen. The frozen liver was thawed to about 0°C , pressed

through a plastic tube with 1 mm bottom holes to remove connective tissue, mixed by stirring and refrozen (19). The procedure was performed in a cold room.

Chemical analysis

The acid soluble fraction and RNA were extracted according to Munro and Fleck (20). We have adopted the figure of Fleck and Munro that 32 microgram of nucleotides give an extinction of 1.000 at 260 nm (21). DNA was analyzed by the technique of Scott et al as modified by Hinrichs et al (22). The radioactivity was measured in a Packard (Downers Grove, Ill.) Tri Carb CD 300 liquid scintillation system. The acid soluble fraction contains all compounds soluble in 0.1 M cold perchloric acid. This fraction and RNA were calculated as microgram of ultraviolet absorbing substances.

Pathology

Rats not given ^3H -orotic acid were used for histopathological examination. Tumor with surrounding liver was fixed in 4 % formaldehyde, embedded in paraffin, sectioned and stained with hematoxylin-erythrosine.

Statistical analysis

Students' two-tailed t-test was employed on the logarithmic values.

RESULTS

On days one, three and five a delimited tumor was found in the liver in all rats. There was more necrosis in the central parts of tumors in rats treated with HAL than in the controls. The tumor has no capsule (Fig 1).

On day ten the HAL rats had still a defined tumor while the control rats had widespread tumor growth in the liver and the peritoneum. In the control rats there were small necroses in the liver immediately surrounding the main tumor, only detectable with microscopic examination. Therefore biochemical studies were not performed in those animals.

The biochemical examinations of the tumors showed a decrease in the content of nucleotides and RNA/g tissue and RNA/microgram DNA on days one, three and five and in acid soluble fraction/microgram DNA on days one and three in the tumor in rats treated with HAL compared with controls (Table I). There were no differences between controls and rats treated with HAL on these days with respect to the labelling of the acid soluble fraction and RNA.

In all rats treated with HAL, there was an increase in the amount of RNA/microgram DNA ($p < 0.001$) and /g tissue ($p < 0.001$) and of nucleotides/g tissue in tumors on day ten compared with day five. The ratio of RNA to acid soluble fraction labelling was also increased on day ten in all groups compared to HAL rats on day five. The significance was $0.001 < p < 0.01$ between the intraportal groups and $p < 0.001$ compared to the

other two administration routes. There was a lower labelling of DNA on day 3 in HAL rats compared to controls ($0.01 < p < 0.02$).

As regards the liver there was, on day five, a significantly higher amount of nucleotide and RNA/microgram DNA in HAL rats than in the controls. The ratio of RNA to acid soluble fraction labelling after intraportal administration of ^3H -orotic acid was higher on day ten than on day five in HAL rats ($0.001 < p < 0.01$, Table II). There were no differences in DNA labelling.

DISCUSSION

Our study showed that one, three and five days after HAL there was a decrease in the nucleotide and RNA content of the tumor cells. There was no such decrease in the liver cells. This difference is probably not a reflection of different sensitivity of the cells to the ischemia but more related to the vascular supply which is predominantly arterial in the tumor and portal in the normal liver. Hepatic artery ligation does not expose the normal liver tissue to any significant ischemia, but the tumor is severely damaged with decrease in nucleotides and RNA and increased necrosis. The used tumor model is well established and the margin between tumor and normal tissue is well defined without any capsule (fig 1). The main vascularization is arterial but portal vessels are found in the periphery and some also seem to reach the central part of the tumor (24). Previous studies in rats have shown retardation of tumor growth after HAL (25). This was also found in our study on day 10 when the rats with HAL had a

delimited tumor in the liver, while the controls had widespread tumor growth in the abdominal cavity. On day 10 the nucleotide and RNA content/g tissue and microgram DNA after HAL reached the same values as the control rats at days one to five.

Ten days after HAL tumor RNA/microgram DNA and g tissue was significantly increased compared to the rats sacrificed after five days. The ratio of RNA to the acid soluble fraction labelling was also increased. These results indicate a recovery of the viability of the tumor, probably due to revascularization. This could be established either by the portal vein or by rearterialization. Our labelling experiments may help to answer this question. Orotic acid is a precursor in pyrimidine synthesis and therefore also easily incorporated into RNA. There was a lower labelling of DNA in rats subjected to HAL than in controls on day 3. There was a trend to lower labelling of tumor RNA and DNA on days three and five in both groups. In control rats this might be explained by progressive tumor growth with less accessibility of portal vein blood flow to the tumor, and in the HAL rats, perhaps also by a decreased viability. The results do not support the idea of an increased vascularization of tumor from the portal vein by days three or five. On day 10, however, the tumor RNA labelling was at same level as on day 1 in the HAL rats. This supports the hypothesis of tumor revascularization. If this was of portal origin a higher labelling of tumor RNA and acid soluble fraction might be expected after administration of the precursor by the portal vein. We have namely found that in rats without HAL administration of the RNA precursor by the hepatic artery gives a much higher labelling of liver tumor RNA than administration by the portal vein, a

femoral vein or intraperitoneally (23). In the present experiment a tumor in the HAL rats was revascularized from the portal vein, portal administration of the precursor should give considerably heavier labelling of the tumor than administration by the other routes. However, there was a tendency to higher labelling of tumor RNA and DNA after administration by a femoral vein. This suggests that an arterial revascularization has taken place.

In conclusion, the increased uptake of ^3H -orotic acid into tumor RNA ten days after hepatic artery ligation strongly indicates rearterialization, perhaps explaining why hepatic artery ligation has only a limited and temporary effect on tumor growth. Consequently portal infusion of cytostatic agents is not more effective than systemic administration when given a week or more after HAL. A complete dearterialization of the liver, which was not performed in the present study might, however, give different results. In a recent experimental study, repeated transient dearterialization was shown to prevent collateral vessel formation (26). This is an important observation and is an approach, which should be evaluated in further studies.

In the liver HAL gave almost no changes. This is in accordance with the concept that portal blood supply dominates and protects the liver from ischemia following hepatic artery ligation.

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Table 1. Chemical analysis of tumor, Nt = Nucleotides, ASF = acid-soluble fraction, n = number of animals.

Time	Group	Nt	μg/g tumor		DNA	Nt	μg μg		DNA	ASF	dpm /μg		DNA	dpm/dpm		n
			RNA	DNA			RNA	DNA			RNA	DNA		RNA	DNA	
1 day	control	1.7±.17	6.0±.28	5.90±.26	.29±.03	1.01±.11	372±111	60±23	.57±.22	144±12	3					
	ligature	1.12±.11 ^c	2.72±.21 ^b	6.10±.41	.19±.02 ^c	.46±.06 ^b	381±141	61±29	.57±.25	101±51						
3 days	control	1.7±.07	6.23±.37 ^a	5.97±.26	.29±.01	1.13±.03	176±18	23±5	.24±.04	127±16	3					
	ligature	1.03±.07 ^a	2.41±.33	4.98±.18 ^c	.21±.01 ^a	.48±.01 ^a	315±57	31±8	.06±.03 ^c	91±9						
5 days	control	1.85±.06	6.01±.26	6.21±.42	.30±.03	.97±.07 ^a	144±24	11±3	.18±.11	72±3	3					
	ligature	1.1±.08 ^b	2.23±.27 ^b	5.04±.45 ^c	.28±.05	.12±.05 ^b	306±72 ^c	22±5	.10±.05	69±5						
10 days	portal	1.87±.07	6.58±.11	4.54±.82	.48±.10	1.70±.34	335±78	49±17	.43±.31	140±1	3					
	ligature	2.0±.07	7.1±.11	6.22±.25	.53±.01	1.15±.03	441±41	53±16	.47±.08	153±7						
10 days	tumor	2.02±.07	7.44±.14	6.29±.18	.32±.02	1.19±.04	486±98	88±23	.87±.24	175±10	3					
	control															

a signif. p < 0.001 compared to corresponding control group
b signif. 0.001 < p < 0.01
c signif. 0.01 < p < 0.02

Table 2. Chemical analysis of liver. The same abbreviations as in table 1.

Group	Nt	µg/g liver RNA	DNA	Nt	µg/µg DNA RNA	dpm ASF	10^{-2} / RNA	µg DNA DNA	dpm/dpm RNA/ASF 10^{-5}	n
1 day	control	3.04±.06	2.21±.07	1.38±.06	3.92±.13	233±50	36±7	.77±.17	159±4	6
	ligature	3.21±.12	2.11±.05	1.48±.08	4.11±.11	182±16	34±4	.76±.09	186±9	6
3 days	control	2.75±.06	1.88±.10	1.49±.09	4.26±.23	178±19	25±3	.42±.06	138±6	6
	ligature	2.76±.07	1.49±.06	1.49±.06	4.33±.28	194±20	27±5	.46±.06	137±11	6
5 days	control	2.73±.05	2.21±.07	1.22±.03 ^b	3.21±.10	121±16	16±2	.32±.04	135±6	6
	ligature	2.85±.06	2.06±.03	1.39±.05 ^b	3.71±.14 ^c	171±22	21±3	.36±.04	122±5	7
10 days ligature	portal	2.83±.17	2.09±.03	1.36±.10	3.80±.32	146±9	24±3	.42±.07	163±10	5
	peritoneal	3.07±.05	2.17±.02	1.42±.03	3.66±.07	144±14	18±3	.27±.03	124±5	5
	femoral	2.99±.05	2.51±.10	1.51±.09	3.33±.17	150±7	19±2	.34±.01	128±10	6

^b signif. 0.01 < p < 0.001 compared to corresponding control group.

^c signif. 0.02 < p < 0.01

HEPATIC ARTERY ADMINISTRATION OF DEGRADABLE STARCH MICRO-
SPHERES; II. EFFECTS ON INCORPORATION OF URIDINE AND URACIL
INTO RNA OF AN ADENOCARCINOMA TRANSPLANTED TO RAT LIVER.

H. Teder¹, C. Erichsen², P.I. Christensson³,
P.E. Jönsson², L. Lewné⁴, U. Stenram³.

¹Department of Surgery, Malmö Allmänna Sjukhus, S-214 01
Malmö, Sweden

Departments of ²Surgery and ³ Pathology, University
Hospital of Lund, S-221 85 Lund, Sweden

⁴ Department of Zoophysiology, University of Lund, S-223 62
Lund, Sweden

Running title: Starch microspheres and liver tumor incorpora-
tion of uridine and uracil.

ABSTRACT

Effects of degradable starch microspheres administered via the hepatic artery were examined in rats in which an adenocarcinoma was transplanted into the liver. ^3H -uridine or ^3H -uracil with cold uridine and uracil, respectively, in amounts corresponding to therapeutic doses of these two pyrimidines as fluoro compounds, were administered with or without microspheres. Labelling of the acid-soluble fraction and RNA of tumour, liver, small intestine, spleen, kidney and bone marrow was examined after 3 and 60 minutes after injection. When microspheres were added the specific radioactivity of tumour RNA was significantly higher both at 3 minutes ($p < 0.05$) and 60 minutes ($p < 0.01$) in the rats given uridine, and in rats given uracil higher at 60 minutes after injection ($p < 0.05$). There were no such differences in the labelling of the normal tissues. The results indicate that arterial administration of cytostatic drugs as 5-fluoropyrimidines together with degradable starch microspheres might increase the cytotoxic effect on tumours nourished by the artery.

INTRODUCTION

To improve the therapeutic effect of cytostatic drugs, regional delivery of the drug together with degradable starch microspheres has been suggested (1). In pharmacokinetic studies with hepatic artery injections of degradable microspheres and cytostatic drugs, it has been shown that systemic drug exposure is reduced (2,3,4). Drug retention in the target organ has been demonstrated by labelling techniques (1,5,6).

When nucleic acid precursors were injected into the rat hepatic artery together with microspheres, the microspheres affected neither the liver cell energy charge nor the incorporation of the nucleic acid precursors into nucleic acids of normal liver cells (7).

In this study in rats with an adenocarcinoma of the colon transplanted to the liver, the effect of the degradable microspheres on the incorporation of uridine and uracil into RNA of the tumour was studied.

MATERIAL AND METHODS

The experiments were performed on 51 female rats, weighing 165-205 g. (Wistar S.P.F., Møllegaard, Skonsved, Denmark).

In all animals 1.0×10^6 cells from an N-methyl-N-nitrosoguanidine induced adenocarcinoma of the colon (8) were inoculated directly into the central liver lobe under ether anaesthesia (9). After approximately 10 days, at which time the solitary liver tumour had a diameter of 8-10 mm, the animals were subjected to further studies.

Twenty animals were used to examine the effect of the microspheres on the incorporation of uracil into RNA of tumour, liver, spleen, small intestine, kidney and bone marrow.

Thirty-one animals were used to study the effect of the microspheres on the incorporation of uridine into RNA of the same tissues.

Operative procedure: The animals were anaesthetized with nitrous oxide/halothane.(10). A polyethylene catheter (Intra-medec, PE 50, Clay-Adams, Parsippany, N.J.) was placed into the gastroduodenal artery with the tip towards the origin of the proper hepatic artery (11). The average operation time was 20 minutes.

Injected substances: The degradable starch microspheres (Spherex^R, batch 70635, Pharmacia AB, Uppsala, Sweden) had a mean diameter of 40 μm (range 20-60 μm) and were delivered in

aqueous solution. The microspheres are degraded by amylase. In vitro, at an amylase concentration of 4 μ kat/l half the microsphere mass is dissolved in 12 minutes; at 25 μ kat/l the time needed is 6 minutes.

3 H-uracil (20 Ci/mmol, 1 mCi/ml) and 3 H-uridine (38.7 Ci/mmol, 1 mCi/ml) were delivered from New England Nuclear Co., Boston, MA. Unlabelled uracil and uridine were delivered from Sigma Chem. Co. St. Louis MO. Uracil 6.45 mg/ml was dissolved in 0.05 M Tris-HCl buffer solution, pH 8.8 and then mixed with 3 H-uracil. Uridine 6.45 mg/ml was dissolved in the same buffer and then mixed with 3 H-uridine.

Injections: All injections were made into the hepatic artery in anaesthetized rats.

Uracil (1.5 mg/100g BW) together with 3 H-uracil (0.125 mCi/100g BW) was injected alone or mixed with the microspheres, 12 mg (12).

Uridine (1.5 mg/100g BW) together with 3 H-uridine (0.125 mCi/100g BW) was injected alone or mixed with the microspheres, 12 mg.

The substances were injected over one minute. The volume was 0.8-0.9 ml. Retrograde flow into the common hepatic artery during the injections was prevented by a loop around the vessel.

Sample preparation: The animals were sacrificed 3 or 60 minutes after the injections. The tissues (tumour, liver, small intestine, spleen, kidney and bone marrow from both femurs) were immediately taken out and frozen in liquid nitrogen and then stored at -70°C until analysis.

Extraction and analysis: DNA was analyzed according to Scott et al. (13) as modified by Hinrichs et al. (14). The RNA extractions were made according to Munro and Fleck (15). The preliminary extract obtained with cold perchloric acid is here called the "acid soluble fraction (ASF)".

Radioactivities were measured in a liquid scintillation spectrometer (Packard 300 CD, Downers Grove, Ill).

Student's two tailed t-test was employed on the logarithmic values. Probability levels are represented by the following signs: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

RESULTS

Uridine injections: In tumour tissue the radioactivity of the acid soluble fractions at both 3 and 60 minutes after injection was higher when microspheres were added ($p < 0.05$) (table 1). The specific radioactivity of RNA in tumour tissue was significantly higher both at 3 minutes ($p < 0.05$) and at 60 minutes after injection ($p < 0.01$) (table 1).

The radioactivity of the acid soluble fraction in the normal liver tissue at 3 minutes after injection was less when uridine was injected with microspheres ($p < 0.05$). At 60 minutes after injection the radioactivity of the acid soluble fractions was equal in the two groups. The specific radioactivity of RNA did not differ significantly between animals given uridine alone or together with microspheres, neither at 3 nor at 60 minutes after injection (table 1).

In the kidney no significant difference was found in the radioactivities of the acid soluble fraction and RNA in animals given uridine alone or with the microspheres, neither at 3 nor at 60 minutes after injection (table 1).

In the other tissues analyzed, the radioactivities of the acid soluble fraction and RNA were equal in animals given uridine alone or with microspheres both at 3 and 60 minutes after the injections.

Uracil injections: In tumour tissue the radioactivity of the acid soluble fraction did not differ significantly between ani-

mals given uracil alone or with microspheres, neither at 3 nor 60 minutes after injection (table 2). The specific radioactivity of RNA in tumour tissue at 60 minutes after injection was higher with microspheres ($p < 0.05$).

In the normal liver tissue the radioactivity of the acid soluble fraction and RNA at 3 and 60 minutes after injection were equal in animals given uracil only or uracil together with microspheres (table 2).

In the kidney the radioactivity of the acid soluble fraction at 3 minutes after injection was less when uracil was injected together with microspheres ($p < 0.05$) (table 2).

The radioactivities of both the acid soluble fraction and RNA of spleen, small intestine and bone marrow were low both at 3 and 60 minutes after injection, with no difference found between animals given uracil alone or together with microspheres.

DISCUSSION

Administration of uridine with degradable starch microspheres via the hepatic artery gives a significantly higher incorporation into tumor RNA than when given without microspheres ($p < 0.01$) (Table 1). With uracil as a test substance the difference was significant at 60 min after injection ($p < 0.05$) (Table 2). In tumour at 3 min after injection the radioactivities in the RNA was unreasonably high (c.f. the activities found at 60 min after injection) possibly due to precipitation of uracil as this substance is very little soluble at acid and neutral pH.

In a similar study with uracil and orotic acid (the latter substance injected in tracer amounts) in rats without tumour there were no differences in the incorporation into liver RNA between the groups with and without microspheres (7). In liver RNA the same result was obtained in this study.

The difference between tumour and normal liver tissue regarding the incorporation of uridine and uracil into RNA can be explained by the predominant arterial vascular supply in the tumour (16,17,18). These observations suggest that co-administration of microspheres gives a retention of RNA precursors in tumour under these conditions. As the vascular supply to many human liver tumours is also predominantly arterial (19) we plan to examine if tumour uptake of such RNA precursors as 5-fluoropyrimidines might also be increased by co-administration of degradable starch microspheres via the hepatic artery. The cytostatic effect of 5-fluoropyrimidines is namely due to its incorporation into RNA and interference with DNA synthesis.

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	DPM in ASF $\mu\text{g DNA} \times 10^{-5}$	Inj. substances	Tumour	Liver	Kidney	Small intestine	Bone Marrow	t_p test
4 MINUTES AFTER INJ.	10^{-5}	uridine	$n=6$ 4.70 $\pm$ 0.84	$n=6$ 7.87 $\pm$ 0.76	$n=6$ 2.42 $\pm$ 0.41	$n=6$ 0.55 $\pm$ 0.17	$n=6$ 0.22 $\pm$ 0.038	$n=6$ 0.294 $\pm$ 0.080
		uridine + DSM	$n=6$ 8.73 $\pm$ 1.05 *	$n=6$ 11.31 $\pm$ 0.42 *	$n=6$ 3.06 $\pm$ 0.15	$n=6$ 0.401 $\pm$ 0.147	$n=6$ 0.294 $\pm$ 0.047	$n=6$ 0.294 $\pm$ 0.047
		uridine	$n=6$ 0.013 $\pm$ 0.002	$n=6$ 0.124 $\pm$ 0.003	$n=6$ 0.014 $\pm$ 0.002	$n=6$ 0.019 $\pm$ 0.004	$n=6$ 0.015 $\pm$ 0.004	$n=6$ 0.012 $\pm$ 0.001
		uridine + DSM	$n=6$ 0.025 $\pm$ 0.003 *	$n=6$ 0.101 $\pm$ 0.003	$n=6$ 0.011 $\pm$ 0.002	$n=6$ 0.011 $\pm$ 0.003	$n=6$ 0.015 $\pm$ 0.004	$n=6$ 0.015 $\pm$ 0.001
60 MINUTES AFTER INJ.	10^{-5}	uridine	$n=6$ 3.86 $\pm$ 0.48 *	$n=6$ 3.23 $\pm$ 0.36	$n=6$ 1.34 $\pm$ 0.07	$n=6$ 0.516 $\pm$ 0.129	$n=6$ 0.137 $\pm$ 0.009	$n=6$ 0.178 $\pm$ 0.015
		uridine + DSM	$n=11$ 6.16 $\pm$ 0.59 *	$n=11$ 3.31 $\pm$ 0.30	$n=11$ 1.38 $\pm$ 0.09	$n=11$ 0.303 $\pm$ 0.021	$n=11$ 0.133 $\pm$ 0.009	$n=11$ 0.183 $\pm$ 0.014
		uridine	$n=8$ 0.025 $\pm$ 0.005	$n=8$ 0.073 $\pm$ 0.005	$n=8$ 0.057 $\pm$ 0.003	$n=8$ 0.056 $\pm$ 0.006	$n=8$ 0.022 $\pm$ 0.003	$n=8$ 0.006 $\pm$ 0.003
		uridine + DSM	$n=11$ 0.087 $\pm$ 0.020 **	$n=11$ 0.080 $\pm$ 0.004	$n=11$ 0.059 $\pm$ 0.004	$n=11$ 0.046 $\pm$ 0.004	$n=11$ 0.024 $\pm$ 0.004	$n=11$ 0.006 $\pm$ 0.001

Table 1 : Rat with an adenocarcinoma transplanted to the liver. Incorporation of uridine into the ASF and RNA of tissues 3 and 60 minutes after hepatic artery administration of uridine (1.5 mg/100 g BW) alone or mixed with degradable starch microspheres (DSM), 12 mg. Mean values $\pm$ S.E.M. Statistical significances; ** = $p < 0.01$, * = $p < 0.05$.

	Inj. substances	Tumour	Liver	Kidney	Small intestine	Bone marrow	Spleen
3 MINUTES AFTER INJ.	uracil	n=5 10.02 ± 2.13	4.04 ± 0.36	3.24 ± 0.24	0.459 ± 0.054	0.255 ± 0.010	0.467 ± 0.021
	uracil + DSM	n=6 15.24 ± 2.44	4.40 ± 0.56	2.41 ± 0.17*	0.590 ± 0.023	0.214 ± 0.019	0.344 ± 0.049
	uracil	n=5 0.021 ± 0.005	0.010 ± 0.001	0.006 ± 0.001	0.0006 ± 0.0002	0.0014 ± 0.0003	0.0003 ± 0.0001
	uracil + DSM	n=6 0.028 ± 0.005	0.009 ± 0.001	0.004 ± 0.001	0.0004 ± 0.0001	0.0012 ± 0.0002	0.0001 ± 0.0001
60 MINUTES AFTER INJ.	uracil	n=4 4.48 ± 1.36	4.06 ± 1.54	0.90 ± 0.09	0.143 ± 0.012	0.107 ± 0.003	0.189 ± 0.014
	uracil + DSM	n=5 7.89 ± 1.52	4.45 ± 1.50	0.89 ± 0.07	0.158 ± 0.011	0.109 ± 0.006	0.207 ± 0.009
	uracil	n=4 0.013 ± 0.003	0.036 ± 0.002	0.007 ± 0.003	0.0037 ± 0.0006	0.0021 ± 0.0001	0.0008 ± 0.0001
	uracil + DSM	n=5 0.024 ± 0.004 *	0.039 ± 0.007	0.007 ± 0.001	0.0035 ± 0.0003	0.0018 ± 0.0001	0.0009 ± 0.0002

Table 2 : Rat with an adenocarcinoma transplanted to the liver. Incorporation of uracil into the ASF and RNA of tissues 3 and 60 minutes after hepatic artery administration of uracil (1.5 mg/100g BW) alone or mixed with degradable starch microspheres (DSM), 12 mg. Mean values ± S.E.M.. Statistical significances; ** = p<0.01, * = p<0.05.

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